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THERMAL INACTIVATION OF COXIELLA BURNETII IN MILK PASTEURIZATION

John B. Enright, et al

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Thermal Inactivation Of Coxiella burnetii In Milk Pasteurization

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Thermal Inactivation Of Coxiella burnetii And Its Relation To Pasteurization of Milk

John B. Enright, Ph.D.
Walter W. Sadler, D.V.M.
Robert C. Thomas, M.S.

Public Health Monograph No. 47

I

The Authors

Dr. Enright and Dr. Sadler are members of the department of public health, School of Veterinary Medicine, University of California, Davis, Calif. Mr. Thomas is a bacteriologist, Milk Sanitation Research Section, Robert A. Taft Sanitary Engineering Center, Division of Sanitary Engineering Services, Public Health Service, Cincinnati, Ohio.

Before coming to Davis, Dr. Enright was with the Viral and Rickettsial Diseases Laboratory, California State Department of Public Health. Dr. Sadler came to Davis from Texas, where he had served as a State milk sanitarian, a city veterinarian, and had been in private practice. Mr. Thomas was assigned to the Q fever study from the Robert A. Taft Sanitary Engineering Center. He has served with the Public Health Service since 1929, and has devoted several years to studies of organisms to be used in testing the efficiency of pasteurizing equipment.

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Public Health Monograph No. 11, "Thermal Inactivation of Coxiella burnetii in Milk Pasteurization," should be corrected as shown below.

Page 18 - In Table 10 the S and N designating survival and first positive sample should not carry the suffix number as the figures in these columns represent period of thermal liberation as designated in the last two columns and not time in minutes as in the other columns.

Page 25 - In Figure 5 the positive point shown to the extreme right of the chart at about .25% minutes should be a negative point as shown in Figure 6.

Page 26 - Line three, column 2 should read to an equivalent holding time of (see table number 4) 63 seconds at a flow rate of 3,30[illegible] lbs. per hour and to an equivalent holding time of 0.21 minutes or [illegible] seconds at a flow rate of 860 lbs. per hour at 161°F. In line 5, same column, substitute pounds per hour for gravity.

Foreword

In recent years, a significant number of human cases of Q fever have occurred in the United States, some of which have been attributed to the consumption of milk. Upon investigation, it was ascertained that Q fever in cattle, goats, and sheep is endemic in certain western and southwestern States. It was also observed that the danger of transmission of the disease to man could be reduced by slight changes in the procedures for pasteurizing milk.

Because of the need for precise information as to the pasteurization times and temperatures necessary to assure destruction of the Q fever organism, the Public Health Service, in cooperation with the University of California, established a research project to study the thermal destruction of this organism. The project, a joint undertaking of the Communicable Disease Center and the Division of Sanitary Engineering Services, both components of the Bureau of State Services, and the University of California's School of Veterinary Medicine, has now been completed.

After reviewing the findings of this study, the Public Health Service notified the dairy industry and State milk control authorities that thermal destruction of the Q fever organism appeared to be achieved when certain time and temperature combinations were maintained throughout the milk pasteurization procedure.

Details about the research project and the new pasteurization recommendation which resulted from it are described in this monograph. The report is presented with the hope that it will prove a valuable reference document for public health officials, for members of the medical and veterinary professions, and for State and municipal officials who are concerned with formulating and enforcing milk sanitation regulations.

Otis L. Anderson
Assistant Surgeon General
Chief, Bureau of State Services

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I. Introduction

The organism of Q fever, *Coxiella burnetii*, is rather widely distributed throughout the world. In the relatively short period of time since 1937, when Derrick (*1*) first described the disease, the organism or the disease it causes has been identified in many countries of the world. An excellent review of the world distribution of Q fever and the isolation of *C. burnetii* from humans, animals, and arthropods has been published by Berge and Lennette (*2*). Another excellent review of the literature has been published by Wentworth (*3*).

In the United States, epidemiological investigations have revealed that cows, sheep, and goats are the important sources of infection for man (*4–9*). Arthropods naturally infected with *C. burnetii* have been found in the United States (*10–12*) but they have not been shown to be of importance in the transmission of the organism from animals to man. *C. burnetii* has been found to be shed in the milk (*8, 13–15*), in other secretions and in excretions (*16–20*) of cows, sheep, and goats. Since the organism is shed by these animals in great numbers, man's environment becomes easily contaminated and he may be exposed either directly, as by drinking infected milk, or indirectly, as by inhalation of contaminated dust or other particles (*21–23*).

Early in the study of Q fever in California, it became apparent that *C. burnetii* was relatively more thermal-resistant than other organisms pathogenic for man, excluding the spore-formers. This first became evident with the isolation of the organism from milk pasteurized according to recommended minimum standards (*24–27*). The rickettsia was also shown to be in whole milk and in the butter and buttermilk from unpasteurized milk of naturally infected dairy cows. The butter was still infectious for guinea pigs 41 days after preparation (*28*).

The director of the California State Department of Public Health made inquiry as to the feasibility of a study to determine the times and temperatures necessary in the pasteurization of milk to eliminate viable *C. burnetii*. As a result, a cooperative project by the University of California and the Public Health Service was undertaken in which the dairy industry supplied both financial and technical support. The project was implemented by an agreement between the Public Health Service and the University of California which specified that the work should include a determination of the thermal deathpoint curve of *C. burnetii* in milk, within the time and temperature ranges utilized in commercial pasteurization processes. It was further specified that the project should incorporate studies on a commercial scale, utilizing pasteurization equipment designed and operated in conformance with the Milk Ordinance and Code 1953 recommendations of the Public Health Service (*29*) as well as with the directions of the manufacturer.

The study was conducted in the department of public health, School of Veterinary Medicine, University of California, Davis, Calif. The participating agencies of the Public Health Service were the Milk and Food Program and the Robert A. Taft Sanitary Engineering Center, both components of the Division of Sanitary Engineering Services, and the Communicable Disease Center, which was the major financial contributor. Dairy Industries Supply Association, Inc., loaned the commercial processing equipment used in the study. Additional funds for the project were obtained from the Milk Industry Foundation, Inc., and from Public Health Service research grant G–4076.

At the beginning of the study, it was realized that certain fundamental questions must be answered before a realistic thermal-resistance study could be conducted. One of these questions concerned the maximum number of *C. burnetii* that might be found in the milk of an infected cow. The determination of this number was an extremely critical point in the

study since the number of organisms in the milk has definite bearing on the amount of heat required for their complete destruction. Several lines of investigation were used to obtain fundamental information on this point. Milk supplies of three communities were sampled; samples of milk were obtained from widely separated areas in California; the milk of dairy cows within an infected herd was sampled at intervals, during which time some of the animals calved; and a dairy cow was infected and the number of rickettsia in her milk was determined. In addition, information was obtained from investigators in other parts of the United States.

Another fundamental question concerned the development of the most sensitive method for determining the presence of small numbers of viable *C. burnetii* in pasteurized milk. Q fever is a well-defined disease of man only; in animals it is an infection without constant clinical or pathological findings, and the causative organism will not grow in artificial media. Therefore, the criterion for infection in the experimental host is the development of specific complement-fixing antibody some 3 weeks after inoculation. The point was raised as to the possibility of heat-killed rickettsiae also inducing the formation of this antibody. Information on this point was obtained by comparing and evaluating several different methods of demonstrating the presence of *C. burnetii*.

After obtaining information on these fundamental questions, a thermal-resistance study with *C. burnetii* suspended in whole raw milk was conducted in the laboratory. This study was followed by the application of these laboratory findings to the pasteurization of milk, using standard commercial conditions, equipment, and controls.

The purpose of this monograph is to report the results of the project and to present the conclusions and recommendations of a study committee relative to providing adequate minimum standards for the pasteurization of milk.

II. Coxiella burnetii in California Milk Supplies

It seems to be generally accepted among investigators of Q fever that the dairy cow is the dominant source of infection for man in southern California, whereas sheep and goats occupy this role in northern California. Furthermore, contamination of the environment by the infected animal rather than its milk probably constitutes the greater hazard for man. This assumption is substantiated by a number of investigators (*6, 8, 9, 30, 31*). These investigations tend to create a sense of security toward the milk supply of northern California although Clark, Lennette, and Romer (*30*) reported that in northern California 82 of 350 persons with Q fever drank raw milk; however, all but 6 of these also had direct contact with cattle or sheep. Lennette and Clark (*32*) do report that samples of pooled raw milk from one area of northern California contained *Coxiella burnetii*.

Serologic surveys by Shepard (*33*) indicate that *C. burnetii* is distributed in the bovine species throughout the western States. Similar surveys by Lennette and associates (*7*) indicate that *C. burnetii* infection is present in the dairy herds of both northern and southern California. Although the infection is not so prevalent in northern California, these investigators do suggest that in two areas an epidemiological pattern similar to that in southern California may exist.

In this section are reported the findings of two surveys in which the occurrence of *C. burnetii* in samples of the milk supply of California was investigated. In the first survey, 109 samples of raw milk going to pasteurization plants in three cities of northern California were obtained from routine specimens collected by the local health departments.

In the second survey, samples of retail milk

Table 1. Results of complement fixation tests for *Coxiella burnetii*, raw-to-plant wholesale milk samples, from the milk supply of three California cities

City of destination	County of origin	Dairies		Positive specimen No.	Results of complement fixation test			
		Number tested	Number with positive samples		Guinea pig No. 1		Guinea pig No. 2	
					Before inoculation	After inoculation	Before inoculation	After inoculation
1	Yolo	5	1	434	<1:8	1:256	<1:8	1:256
2	Yolo	10	1	1120	<1:8	1:64	<1:8	<1:8
3	Solano	15	0					
3	Sacramento	67	1	2302	<1:8	1:128	<1:8	1:128
3	Yolo	6	0					
3	Butte	2	1	2736	<1:8	1:128	<1:8	1:128
3	Glenn	3	0					
3	San Joaquin	3	2	2210	<1:8	1:64	<1:8	Died
				2572	<1:8	1:128	<1:8	1:128
3	Sutter	10	1	2233	<1:8	1:128	<1:8	1:64
3	Stanislaus Merced	1	1	[1] 2730	<1:8	1:128	<1:8	1:128
3	Amador	4	0					
3	Placer	1	0					
3	Yuba	1	0					
3	Tehama	1	0					
Total		1089	8					

[1] This sample was taken from a tank truck containing the milk from 2 farms in 1 county and 1 farm from another county.

were obtained from specimens submitted for competition at the California State Fair in August 1951. There were 208 pasteurized milk, 126 pasteurized cream, 37 raw milk, and 5 raw cream samples obtained and tested for *C. burnetii*. In this survey, an attempt was made to determine the number of rickettsiae in the positive milk samples in terms of the highest dilution of milk that would infect guinea pigs.

Materials and Methods

Milk Samples. Samples of milk were collected in sterile containers and immediately transported to the laboratory, where they were kept frozen at −10° C. until guinea pigs became available for inoculation. In preparation for inoculation, each sample was mixed with 200 units of penicillin and 20 mg. of sulfadiazine per milliliter of milk and allowed to remain in the refrigerator at +4° C. for 1 hour. Two-milliliter quantities of each sample were inoculated intraperitoneally into each of two guinea pigs.

Guinea Pig Serums. All guinea pigs were bled before inoculation and again 3 weeks after inoculation. The preinoculation and postinoculation paired serums were tested by complement fixation. The details of this test are given in section IV. Cages containing normal guinea pigs were placed at random throughout the animal house. These guinea pigs were bled at frequent intervals and their serums tested for evidence of experience with *C. burnetii*. To be considered to have experienced antigenic stimulation by *C. burnetii*, an animal must show the appearance of a significant complement-fixing titer between the preinoculation and postinoculation blood serums. It was assumed that a guinea pig had been accidentally exposed to *C. burnetii* if its preinoculation serum was negative in a dilution of 1:8, the lowest dilution used in the test, and its postinoculation serum was positive in the 1:32 dilution or higher.

In the survey of retail milk collected at the State fair, all positive samples were subsequently prepared in tenfold dilutions in sterile skim milk, and 2 ml. of each dilution was inoculated intraperitoneally into each of six guinea pigs. Complement fixation tests were run on the preinoculation and postinoculation serums from these animals. In this way, the titer of

425000—52——2

each sample was determined in terms of infective guinea pig doses.

Results

The results of testing the 109 samples of raw milk from the milk supply of three northern California cities for the presence of *C. burnetii* are presented in table 1. The total number of samples is listed for each city according to the county of origin of the samples. Listed also are the number of positive samples from each county, the laboratory number of the positive milk specimen, and the preinoculation and postinoculation complement-fixing (CF) titer of the two guinea pigs used in each case.

Each milk sample was obtained from the milk of a different dairy. One of the positive samples (No. 2750) was obtained from a tank truck that contained the milk from 3 large dairies, 2 located in one county and 1 in an adjacent county. In two instances (samples Nos. 1120 and 2210), rises in titer were demonstrated in only one guinea pig. In one case, the second pig died before postinoculation bleeding, and in the other, the second animal failed to develop antibody. None of the normal guinea pigs in cages alongside the inoculated animals developed demonstrable complement-fixing antibody to *C. burnetii*.

Table 2 presents the results of testing the 376 retail milk or cream samples from the California State Fair. For raw samples, results are given according to type of product and county of origin; for pasteurized samples, according to county where the milk was pasteurized.

The 376 retail milk samples were selected from milks submitted to the California State Fair in competition for highest score. Among other things this scoring includes points for low

Table 2. Results of testing 376 retail milk samples [1] for the presence of *Coxiella burnetii*

County of origin	Cow's milk								Goat's milk			
	Pasteurized				Raw				Pasteurized		Raw	
	Milk		Cream		Milk		Cream					
	Number samples	Number positive	Number samples	Number positive	Number samples	Number positive	Number samples	Number positive	Number samples	Number positive	Number samples	Number positive
Los Angeles	108	0	61	[2] 1	10	4	2	1			1	0
San Diego	37	0	29	0	7	5	1	1				
Santa Barbara	5	0	3	0								
Orange	6	0	3	0								
Tulare					1	1						
Fresno	13	0	9	0	3	2	1	0				
Sacramento	5	0	2	0							2	0
San Joaquin			2	0					1	0	1	0
Solano	5	0	5	0	1	0						
Yolo	2	0	1	0	1	0						
Contra Costa									1	0	1	0
Glenn	1	0										
El Dorado			1	0	1	0	1	0				
San Francisco			5	0								
Santa Clara	9	0	1	0	2	0						
Napa	2	0	2	0	1	0						
Humboldt	2	0										
Shasta	3	0										
Siskiyou	3	0	2	0	4	0						
Modoc					1	0						
Klamath (Oregon)	5	0										
Total	206	0	126	1	32	12	5	2	2	0	5	0

[1] From the California State Fair.

[2] This is a half-and-half product pasteurized by the high-temperature short-time (HTST) method. The phosphatase test was negative.

Table 3. Infectivity for guinea pigs of dilutions of positive retail milk samples

County of origin	Type of product	Sample No.	Results of guinea pig inoculations [1]					Demonstrated end point
			Undiluted	10^{-1}	10^{-2}	10^{-3}	10^{-4}	
Los Angeles	Half-and-half	5021	1/2	0/4	0/6	0/3	0/5	Undiluted.
Los Angeles	Raw milk	4486	1/2	3/6	0/5	0/6	0/4	10^{-1}.
Los Angeles	do	4665	2/2	5/6	3/5	0/6	0/6	10^{-2}.
Los Angeles	do	4736	1/2	1/6	0/5	0/6	0/6	10^{-1}.
Los Angeles	do	4937	1/2	2/6	0/6	1/6	0/5	10^{-1}.
Los Angeles	Raw cream	5031	1/2	0/6	0/6	0/6	0/6	Undiluted.
San Diego	Raw milk	4451	2/2	1/6	1/6	0/6	0/5	10^{-2}.
San Diego	do	4496	1/2	4/6	3/6	0/5	0/5	10^{-2}.
San Diego	do	4515	2/2	4/6	1/5	0/6	0/5	10^{-2}.
San Diego	do	4530	1/1	2/5	0/4	0/6	0/5	10^{-1}.
San Diego	do	4542	2/2	2/6	1/5	0/6	0/6	10^{-2}.
San Diego	Raw cream	4694	2/2	2/5	2/6	1/5	0/6	10^{-3}.
Fresno	Raw milk	4768	1/1	0/5	0/6	0/6	0/6	Undiluted.
Fresno	do	4769	1/1	3/5	2/5	1/6	0/5	10^{-3}.
Tulare	do	5053	2/2	6/6	6/6	0/6	0/6	10^{-2}.

[1] Numerator equals number of guinea pigs showing fourfold rise or more in CF titer to *C. burnetii;* denominator, number of pigs inoculated.

bacterial count, and these samples are presumably from the best products produced by each creamery. Furthermore, since relatively little raw milk is sold in California, the survey is weighted in favor of pasteurized milk samples. The first four counties listed—Los Angeles, San Diego, Santa Barbara, and Orange—are in southern California; the rest are in central and northern California and represent counties situated in coastal, valley, foothill, and mountainous areas.

In table 3 are presented the infectivity titers for guinea pigs of each positive retail sample of milk or cream, by county of origin. The infectivity titer is calculated on the basis of the highest tenfold dilution in which one or more of the inoculated guinea pigs showed a significant rise in antibody titer to *C. burnetii*.

Figure 1 shows the counties from which samples of unpasteurized milk collected in both surveys were demonstrated to contain the organism of Q fever.

Discussion

The milk supply of each of three surveyed cities in the milksheds of northern California was contaminated with *C. burnetii*. Table 1 shows that 1 in 5, 1 in 10, and 1 in 13 herds supplying the respective cities contained *C. burnetii* in demonstrable numbers. Eight, or 7.3 percent, of 109 raw-to-plant samples contained viable rickettsiae. One sample was taken from a tank truck containing the pooled milk of three large dairies, indicating that either a large majority of the cows of each dairy were infected or that a few cows were shedding very large numbers of organisms. A relatively large proportion of the dairies supplying these communities were sampled. Each milk sample included in this survey was obtained from a different dairy.

Table 2 demonstrates the relative percentage of raw milk and of pasturized milk that contained *C. burnetii*. Only one pasteurized product was positive, and this was half and half cream. On a percentage basis, the relationship is 33.3 percent of the raw milk versus 0.3 percent of the pasteurized milk.

Table 2 also shows that raw milk from each of five goat dairies was negative for *C. burnetii*. Inasmuch as the purpose of the survey reported in table 2 was to ascertain the maximum number of organisms likely to be found in market milk, and inasmuch as the milk of southern California was known to be heavily contaminated with *C. burnetii*, a greater proportion of available time and supplies was devoted to milk from this area. The data presented are, therefore, weighted in favor of southern California. The number of samples collected is not presented as a representative sampling of the retail milk supply of California as a whole but only as the largest number of retail milk samples that have been examined for *C. burnetii* to

date. Furthermore, it should be pointed out that, in this survey, each sample is from a different creamery, and each sample, therefore, is a composite of samples from different producers.

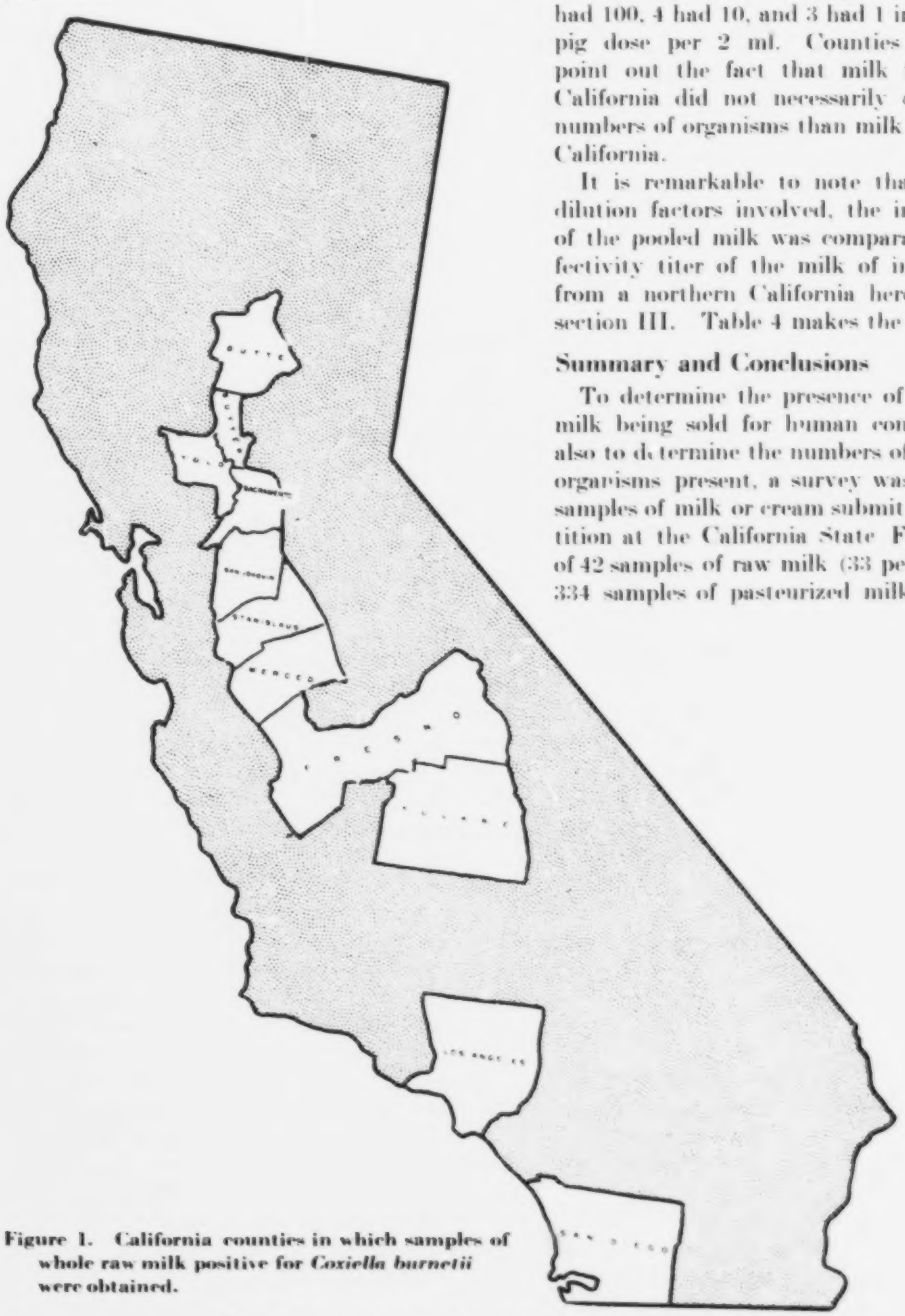

Figure 1. California counties in which samples of whole raw milk positive for *Coxiella burnetii* were obtained.

Table 3 lists the titration results of the 15 milks found positive in the survey of retail market milk and shows that the maximum dilution infectious for guinea pigs in this survey was 10^{-3}. Of these samples, 2 had 1,000, 6 had 100, 4 had 10, and 3 had 1 infective guinea pig dose per 2 ml. Counties are listed to point out the fact that milk from southern California did not necessarily contain higher numbers of organisms than milk from northern California.

It is remarkable to note that, due to the dilution factors involved, the infectivity titer of the pooled milk was comparable to the infectivity titer of the milk of individual cows from a northern California herd described in section III. Table 4 makes the comparison.

Summary and Conclusions

To determine the presence of *C. burnetii* in milk being sold for human consumption and also to determine the numbers of demonstrable organisms present, a survey was made of 376 samples of milk or cream submitted for competition at the California State Fair. Fourteen of 42 samples of raw milk (33 percent) and 1 of 334 samples of pasteurized milk (0.3 percent)

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Table 4. Comparison of infectivity titers of pooled and individual milk samples

Sample	Highest infective dilution				Total samples
	10^{-3}	10^{-2}	10^{-1}	Undiluted	
Individual cow's milk.	3	5	5	5	18 cows.
Pooled market milk.	2	6	4	3	15 pools.

contained viable *C. burnetii*. Of the 15 positive milk samples, 2 contained 1,000, 6 contained 100, 4 contained 10, and 3 contained 1 infective guinea pig dose of *C. burnetii* per 2 ml. It must be remembered that these were samples of retail milk and that each sample presumably was made up of the pooled milk of several cows. These facts would seem to indicate either a very high percentage of shedders of *C. burnetii* or a very high number of organisms in the milk of a few animals.

A survey was made of wholesale milk going to three northern California cities for pasteurization to determine the distribution of *C. burnetii* in the milksheds of those areas. This survey showed that the milk supply of all three cities contained viable *C. burnetii* in demonstrable numbers. Eight of 109 samples were positive. Each sample was obtained from a different producer. If these are considered to be representative samples, then 1 of 5, 1 of 10, and 1 of 13 of the dairies supplying the respective cities were shipping milk containing the organism of Q fever.

Quantitative determination of the infective titer of positive samples of retail milk indicated that no difference existed between samples obtained in northern and in southern California.

C. burnetii was found in 22 raw milk samples collected from 10 counties and, although this is in no way a representative sampling of the milk of California, it is pointed out that the probability of the milk supply of any California city being contaminated is very real.

III. Shedding of Coxiella burnetii in the Milk of Infected Dairy Cows

No information was found in the literature as to the number of organisms shed in the milk of cows naturally infected with *Coxiella burnetii* or as to the possible relationship of these numbers of organisms to antibody levels. Luoto and associates (*34*) have reported that 90 to 95 percent of lactating cows with complement-fixing antibody titers of 1:32 or higher have been found to be shedding *C. burnetii* in their milk.

As reported in section II, the greatest number of Q fever organisms found in the 376 milk samples submitted to the California State Fair was in the order of the number contained in 1,000 infective guinea pig doses of *C. burnetii*.[1] The samples of milk were pooled and presumably contained the milk from both shedders and nonshedders of the rickettsiae. The infective titer obtained indicated that either all the cows whose milk made up a pool were shedding equal numbers of organisms in their milk or that some animals were shedding tremendous numbers of organisms. It was also suggested that the milk from an infected cow might contain a different number of organisms at different times and that the greatest number of organisms might be found soon after the cow had freshened. It had been demonstrated that 100 million infective guinea pig doses per milliliter might be found in the placental tissues and fluids of infected dairy cows (*20*).

Before starting the Q fever pasteurization studies it was necessary to investigate further the number of organisms in a sample of milk in

[1] An infective guinea pig dose may be defined as the minimum number of organisms required to infect a guinea pig by intraperitoneal inoculation. In this study these organisms were always contained in 2 ml. of the inoculum.

order to determine the rickettsia population which should be subjected to heat.

Several investigators were asked about the results of their studies of the infectivity titer of cow's milk. The highest titer reported was 10,000 infective guinea pig doses per milliliter of milk. Some of the milk samples in which the investigators found this number of rickettsiae had been frozen and thawed, a procedure that might kill some of the organisms.

A lactating dairy cow was inoculated by way of the teat canal with the Henzerling strain of *C. burnetii*. She was nursing two calves, and presumably was milked out each day. On the ninth day after she was inoculated a milk sample was taken, diluted, and inoculated into guinea pigs without freezing. The sample of milk contained 10,000 infective guinea pig doses per milliliter.

This section presents the results of a study of individual dairy cows within an infected herd.

Materials and Methods

The Dairy Herd. The dairy herd investigated was composed of approximately 200 animals and was operated by and for a northern California mental institution. This was not a closed herd; animals were brought in as needed from other State-owned herds in both northern and southern California. The milking string averaged about 140 animals at any given time and only the milking cows were bled. The herd had a history of excessive mastitis and it was for this reason that the original blood samples were taken.

Bovine Blood Specimens. Blood was obtained by the usual jugular vein technique from 137 cows in the milking string on March 26, 1952, and again from 108 of these cows on July 16, 1952. The 29 cows not bled on July 16 were either dry or had been added to the herd since March 26. The specimens were forwarded to the laboratory, where they were tested by complement fixation for antibody to *C. burnetii*. This survey was made by Dr. Lauri Luoto and, through his courtesy, the results were made available to the authors.

Milk Samples. On the evening of October 2, 1952, samples were taken from the milking machine pail at the end of a complete milking of each cow. Twenty-five milliliter samples were poured into sterile screw-top vials and placed immediately in a field ice chest. These were transported to our laboratory in Davis and frozen at −10° C. until inoculated into guinea pigs. Over a period of 60 days, as guinea pigs became available, 2 ml. of each sample were inoculated intraperitoneally into each of two animals. These animals were bled and their serums tested for the appearance of antibody to *C. burnetii* as described in section II.

Results

The results of screening the 137 samples of milk are presented in table 5. The blood serum titers of the cows are those of the July 16 bleeding, 78 days prior to sampling the milk. Eighteen cows, or 13.1 percent of those of each antibody level whose milk was tested, were found to be shedding *C. burnetii* in their milk.

Table 6 lists the blood serum CF titer, the infectivity titer of the milk, and the date of last parturition of the 18 cows found to be shedding *C. burnetii* in their milk. Since the purpose of this study was to obtain information on the greatest number of rickettsiae in cow's milk, infectivity titers listed in table 6 are the highest dilution of infected cow's milk that would induce the formation of antibody in one or more of the guinea pigs inoculated. The milk from 3 of the 18 cows was positive in a dilution of 10^{-3}, from 5 in a dilution of 10^{-2},

Table 5. Results of testing milk from 137 cows in an infected herd for *Coxiella burnetii*, according to blood serum antibody titers

Serum CF titer of cow [1]	Number cows tested	Cows positive	
		Number	Percent
1:64	8	4	50.0
1:48	3	0	.0
1:32	11	5	45.5
1:24	4	0	.0
1:16	16	4	25.0
1:12	2	0	.0
1:8	7	1	14.3
1:6	1	0	.0
1:4	56	2	3.6
N. B.[2]	29	2	6.7
Total	137	18	13.1

[1] 2 bleedings, March 26 and July 16, 1952.
[2] Not bled on July 16, 1952.

Table 6. Blood serum antibody titers, infectivity of milk, and parturition dates of 18 cows found to be shedding *Coxiella burnetii* in their milk on October 2, 1952

Cow No.	Blood serum antibody titer of cow		Infectivity end point of the cow's milk [1]	Date of last parturition
	March 26, 1952	July 26, 1952		
				1952
P920	1:64	1:64	10^{-3}	Feb. 27
874	1:128	1:64	10^{-3}	Apr. 26
R552	1:256	1:64	10^{-3}	Aug. 15
P898	N. T [2]	1:6	10^{-2}	June 14
931	1:32	1:32	10^{-2}	Apr. 23
936	1:256	N. T	10^{-2}	July 17
P822	1:64	1:32	10^{-2}	May
917	N. T	1:16	10^{-2}	Apr. 28
960	1:128	1:32	10^{-1}	May 26
R3	N. T	N. T	10^{-1}	July 31
993	N. T	1:6	10^{-1}	Jan. 28
971	1:32	1:32	10^{-1}	June 28
R18	1:16	1:32	10^{-1}	Apr.
P834	N. T	1:8	Undiluted	Sept. 9
929	1:128	1:64	do	Sept. 14
953	1:128	1:16	do	Jan. 20
949	1:16	1:16	do	Apr. 24
P938	N. T	1:16	do	Mar. 21

[1] Highest dilution of milk in which presence of rickettsiae could be demonstrated.
[2] N. T.: Not tested.

Table 7. Infectivity of milk and parturition dates of four cows naturally infected with *Coxiella burnetii*

Cow No.	Date of first parturition [1]	Infectivity of milk, Oct. 2, 1952 [2]	Date of second parturition	Infectivity of milk, May 5, 1953 [2]
	1952		*1953*	
P920	Feb. 27	10^{-3}	Feb. 5	10^{-3}.
931	Apr. 23	10^{-2}	Apr. 13	Not infectious.
R18	Apr.	10^{-1}	Mar. 15	Do.
			1952	
993	Jan. 28	10^{-1}	Nov. 24	Do.

[1] First parturition observed in this study and not necessarily the animal's first calf.
[2] Highest dilution of milk in which rickettsiae could be demonstrated.

and from 5 in a dilution of 10^{-1}. Five milk samples were positive only when undiluted.

On May 5, 1953, samples of milk were again collected from the only 4 of the original 18 positive cows still in the milking string. The results of this second sampling of milk are shown in table 7. All four cows had calved between milk samplings; however, we could no longer demonstrate *C. burnetii* in the milk of three, while the fourth, No. P920, was shedding as many organisms in her milk as before. *C. burnetii* was isolated from the second sample of milk from cow No. P920 and was adapted for growth in the yolk sac of embryonating chicken eggs. This strain we have designated as the Sonoma-53 strain. In view of the fact that at no time was antibody for *C. burnetii* demonstrated in the serums of the numerous control guinea pigs, the probability of any positive titer being due to cross-infection would seem to be negligible.

Discussion

This dairy herd was not in a proved endemic area but, on the other hand, neither was it a closed herd. The animals were in all stages of lactation and pregnancy and varied in age from 2 to 11 years. They had been in the herd anywhere from a few months to several

years so that it would seem reasonable to assume that their exposure to, and their infection with, *C. burnetii* varied considerably.

Of the 137 animals in the herd, 22 had a serum titer of 1:32 or greater, but only 9 of these 22 were secreting demonstrable numbers of organisms in their milk 78 days later (table 5). These findings do not conform with those of Luoto and associates (*34*); however, it must be remembered that their work was done in an endemic area in which the number of susceptible emigrants is relatively high and milk and serum samples were taken at approximately the same time. Nevertheless, it is of interest to note that the milk of 41 percent of the animals with serum titers of 1:32 or greater contained *C. burnetii*. We did not sample the milk of these animals repeatedly nor determine their serum antibody level at many intervals. Nevertheless, the correlation between serum titers and the shedding of organisms in the milk is good. Recently Luoto and Mason (*35*), and Luoto (*36*) have perfected a capillary tube agglutination test for Q fever that may be used directly on samples of milk. It would be interesting to correlate this test with the actual shedding of *C. burnetii* by the animal.

The number of samples of milk in our survey is small and the length of time that each cow was infected is not known. There is evidence that 12 of the 18 positive cows studied had been infected 200 days before their milk was sampled. No correlation between the number of organisms in the milk, the antibody titer, and the times of parturition is apparent for these 12 cows (table 6).

Table 7 shows that on May 5, 1953, cow No. P920 was still shedding the same high concentration of organisms in her milk as she had been shedding in the milk sample obtained on October 2, 1952, 205 days previously and 405 days after her first positive blood test on March 26, 1952 (table 6). The other animals listed in table 7 could not be shown to be still shedding *C. burnetii* in their milk.

Summary

A milking herd of 137 lactating cows was surveyed to determine the numbers of *Coxiella burnetii* in the milk of individual cows. Eighteen cows were shedding organisms in detectable numbers. The milk from 3 cows contained 1,000 infective guinea pig doses of *C. burnetii* per 2 ml.; the milk from 5 cows, 100 doses; and the milk from 5 cows, 10 doses. The milk from 5 cows would infect guinea pigs in 2 ml. quantities only when undiluted.

One of the cows shedding 1,000 infective guinea pig doses per 2 ml. in her milk was found to be shedding the same number 7 months later after a subsequent calving. One cow shedding 100 infective guinea pig doses of *C. burnetii* and two shedding 10 doses were found to have ceased shedding demonstrable numbers of organisms 7 months later and after a second calving.

Fifty percent of the cows with a serum titer of 1:64, 45.5 percent of those with a titer of 1:32, and 25 percent of those with a titer of 1:16 after 78 days were shedding *C. burnetii* in their milk.

The maximum number of *C. burnetii* demonstrated in the milk of an infected dairy cow was the number of organisms contained in 10,000 infective guinea pig doses of *C. burnetii* per milliliter. However, several titers of 1,000 infective guinea pig doses were found in the pooled product of at least one whole milking string of about 20 cows, and possibly several strings were involved. Furthermore, some of the 10,000 dose titers were obtained by other investigators after the milk sample had been frozen and thawed, a process that may kill some of the rickettsiae. Also, since not all breeds of dairy cattle were tested and since some breeds might shed a greater number of organisms in their milk than the breeds tested, it was deemed prudent for the thermal-inactivation studies to use a test population of *C. burnetii* represented by the number of organisms in 100,000 infective guinea pig doses.

IV. Methods of Demonstrating Viable Coxiella burnetii in Pasteurized Milk

It has been pointed out that Q fever is a well-defined disease in man only. Although *Coxiella burnetii*, the organism of Q fever, infects animals, it is associated with neither characteristic symptomatology nor constant pathological findings. Therefore, the usual method of determining the presence of *C. burnetii* is to inoculate a host with a sample of milk and test blood serum for complement-fixing antibody. However, some concern was expressed by the investigators over the possibility of the killed organisms inducing the formation of antibody, especially in those samples near the end point of infectivity, in which the dead rickettsiae would far outnumber those still viable. *C. burnetii* may also be grown in the yolk sac of embryonating chicken eggs.

Four methods of inoculation were investigated in an attempt to ascertain whether or not one method was more reliable than the others in detecting small numbers of viable rickettsiae. The methods compared were as follows: 1 passage in guinea pigs (GP1), 2 passages in guinea pigs (GP2), 3 passages in embryonating eggs (E3), and 1 passage in guinea pigs followed by 3 passages in eggs (GP1E3). Both heated and unheated samples of milk were used in comparing the four methods. Figure 2 shows the flow of materials followed in each comparison of methods.

In these comparative tests, tenfold dilutions

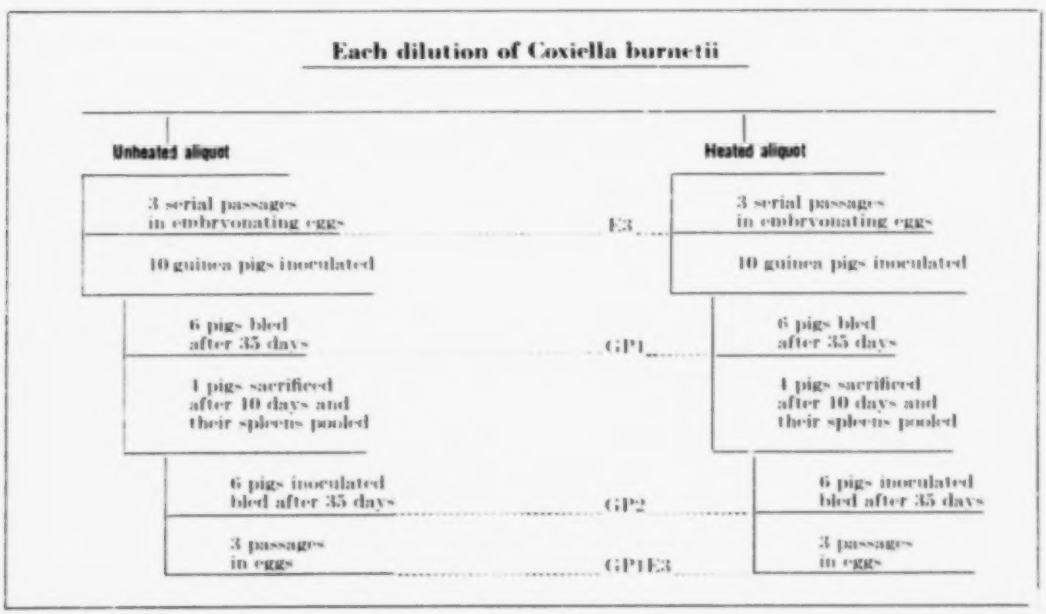
Each dilution of Coxiella burnetii
Unheated aliquot
Heated aliquot
3 serial passages in embryonating eggs
E3
3 serial passages in embryonating eggs
10 guinea pigs inoculated
10 guinea pigs inoculated
6 pigs bled after 35 days
GP1
6 pigs bled after 35 days
4 pigs sacrificed after 10 days and their spleens pooled
4 pigs sacrificed after 10 days and their spleens pooled
6 pigs inoculated bled after 35 days
GP2
6 pigs inoculated bled after 35 days
3 passages in eggs
GP1E3
3 passages in eggs

Figure 2. Protocol for inoculation of guinea pigs in each of four comparative tests of methods of detecting viable rickettsiae

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of the stock suspension of *C. burnetii* were made. Aliquots from each dilution were heated at a given temperature for 30 minutes. Both the unheated and the heated sample of each dilution were inoculated into animals according to the protocol shown in figure 2.

Materials and Methods

Source of C. burnetii. The Henzerling (Italian) strain of *C. burnetii*, in the form of a 20-percent yolk-sac suspension, was maintained in our laboratory by serial passages in embryonating eggs inoculated into the yolk sac. It was our practice to pool the yolk sacs from large numbers of infected eggs, grind them thoroughly in a mortar with alundum, prepare a 10 percent suspension in sterile skim milk, flame-seal 2 ml. aliquots in pyrex tubes, and store them in a CO_2 icebox at $-60°$ C. until needed for use. The infectivity titer of each stock pool so prepared was determined from time to time.

Diluent. Autoclaved skim milk was the standard diluent used in these studies, unless otherwise specified and described.

Inoculation of Guinea Pigs. Healthy guinea pigs of either sex were used. These animals were bled by intracardial puncture approximately 72 hours before inoculation and, unless the animals were sacrificed, a postinoculation blood sample was obtained 30 to 35 days after inoculation. The inoculum consisted of 2 ml. of the sample given by the intraperitoneal route. Guinea pigs that did not survive the period of observation were discarded.

Inoculation of Embryonating Eggs. Fertile hen's eggs that had been embryonating for 5 to 8 days at 100° F. were inoculated with 0.5 ml. of sample directly into the yolk sac. The inoculated eggs were incubated at 95° F. for 8 to 10 days and were candled daily to determine the viability of the embryo. At the end of the postinoculation incubation period the yolk sac from each embryo was harvested. Several impression smears of each yolk sac were stained by Macchiavello's method and examined for the presence of rickettsiae.

Complement Fixation Tests. The complement fixation test was performed using the method developed in the California State Viral and Rickettsial Diseases Laboratory (*8*).

The saline solution used as a diluent for the reagents and in the test proper contained a veronal-bicarbonate buffer as described by Mayer, Croft, and Gray (*37*) and Wallace, Osler, and Mayer (*38*).

The antigen used throughout the study was a commercial preparation of the Henzerling strain of *C. burnetii*. Serial twofold dilutions of the antigen were tested against serial twofold dilutions of a known antiserum. The highest dilution of antigen giving 3+ or 4+ fixation after overnight incubation at 4° C. was taken to represent 1 unit. Two units of antigen were used in the test.

The hemolysin activity of the antigen was determined by titration at 37° C. for 30 minutes with a 1:20 dilution of guinea pig complement and a 2.5 percent suspension of washed sheep red cells. Two units of hemolysin were used in the test. Complement was titrated in the presence of 2 units of antigen at a temperature of 37° C. for 30 minutes. The highest dilution showing complete hemolysis was considered as containing one unit of hemolysin. Two units were used in the test.

Sensitized sheep cells were prepared by mixing together equal parts of a saline solution of hemolysin and washed sheep red cells. Five-tenths milliliter of this 2.5 percent suspension of sheep red cells contained 2 units of hemolysin.

The same final volume of 1.1 ml. suspension was used in both the titrations and the tests. In the test, serum was diluted 1:8 with saline and inactivated for 20 minutes at 60° C. Serial twofold dilutions of serum were then made in saline. Each tube in the test contained 0.2 ml. of complement containing 2 units of hemolysin. This mixture was incubated overnight at 4° C., then placed in a water bath at 37° C. for 10 minutes, after which 0.5 ml. of sensitized cells was added and the tubes returned to the water bath at 37° C. When the appropriate control tubes had cleared, the test was read. Either a 3+ or 4+ fixation was presumed "positive," whereas partial fixation below the 3+ level was considered negative.

The usual controls for the hemolytic system, antigen, anticomplementary effects of the unknown serums, and so on, were included in each test. In addition, normal tissue controls, a

titration of complement activity and of the known antiserum pool, and serum from known normal animals were included in each test.

The preinoculation and postinoculation blood serum samples were run in the same test. A test was discarded and the results were not included in the data if a preinoculation blood sample showed fixation in the lowest dilution, 1:8. A test in which the preinoculation blood was negative in a dilution of 1:8 and the postinoculation blood was positive in a dilution of 1:32 or higher was considered evidence that the guinea pig in question had been specifically exposed to *C. burnetii*.

Control of Cross-Infection. In the animal house, normal, healthy guinea pigs were caged at random. Approximately 1 of every 10 cages contained these controls. The animals were bled at intervals and none were positive throughout the study.

Preparation of Milk Samples. In each test the infectivity titer of both unheated and heated samples of milk was determined. Decimal dilutions of the stock rickettsial suspension of sheep red cells were made in sterile skim milk and each of these dilutions was divided into two equal portions. One series of dilutions was stoppered and placed in the refrigerator, and 10 ml. of each dilution of the other series was flame-sealed in a thin-walled glass ampule and heated. Immediately after heating, 4 or 5 successive dilutions of both the heated and unheated series were inoculated into the host of choice. The dilutions to be inoculated into the hosts were chosen so that infectivity end points would be determined.

Heating Procedure. The samples of milk to be heated were placed in a test tube rack and a carrier device was placed around the neck of each ampule above the milk line. Similar ampules containing the same volume of milk, in which thermocouples extended into the milk and into the airspace above the milk, were also placed in the test tube rack. The rack was then lowered into the hot-water bath, completely submerging the ampules. The rack was vigorously shaken with a mechanical shaker during the heat-up and holding period. The water in this bath was kept at the holding temperature. At the end of the heating period, the proper number of ampules was transferred by means of the holding device to a cold-water bath kept at 40° F., and submerged. The hot-water bath was of large capacity and was equipped with a mechanical stirring device which kept the water moving. The temperature was kept constant by a mercury thermostat with a rated ±0.2° F. accuracy. Two thermometers checked by the Bureau of Standards were placed in this water bath. One was of the maximum-minimum recording type and the other, of the ordinary immersible type. In addition, thermocouples were placed in the water bath in close proximity to the test samples. The thermocouples in the water bath and those in the ampule were connected to a recording device and temperatures were recorded at 3-second intervals on a permanent chart. In this manner a record of the heating-up, the holding, and the cooling-down times of the milk sample and the airspace above it were obtained.

GP1 Inoculation Method. In the GP1 method of determining the passage of viable *C. burnetii* in a test sample of milk, each of six guinea pigs was inoculated intraperitoneally with 2 ml. of the test sample and bled according to the schedule outlined in figure 2.

GP2 Inoculation Method. Each of four guinea pigs was inoculated with 2 ml. of the test sample of milk. Rectal temperatures were taken on these animals daily until experience indicated that the proper time to sacrifice them was on the 9th or 10th day after inoculation. Their spleens were harvested, pooled, ground in a mortar with alundum, and diluted with sterile skim milk to a 10-percent suspension. Each of six guinea pigs was inoculated with 2 ml. of this suspension and bled as described under "Inoculation of Guinea Pigs" (p. 12).

E3 Inoculation Method. Three serial passages of the test sample of milk in embryonating chicken eggs were employed in the E3 method, using the technique described for yolk sac inoculation of eggs. The yolk sacs of the eggs of each passage were examined microscopically for the presence of the rickettsiae. In a few instances, the third passage yolk sacs were inoculated into guinea pigs in an attempt to induce the formation of specific antibody.

GP1E3 Inoculation Method. The GP1E3 method was a combination of the inoculation methods described above. The first passage in

guinea pigs was accomplished as in the GP2 method and the spleen suspension was then passaged three successive times in eggs, as outlined under the E3 method.

Infective Dose. The "infective dose" is used more often in the study of viruses and rickettsiae than in studies of other micro-organisms because viruses and rickettsiae will multiply only within the cells of a susceptible host. Furthermore, the numbers of these organisms required to institute an infection vary greatly, depending on the net result of the aggressiveness of the micro-organism and the defensive powers of the host, and both of these factors may vary considerably. For the purposes of this study, one infective dose (ID) may be defined as the minimum number of *C. burnetii* that will cause infection when suspended in a specified diluent, handled in a routine manner, and inoculated in standardized amounts by a definite route into apparently normal, healthy animals of a given species. Nevertheless, the concept should be kept in mind that the smallest unit of measurement in this study, the infective dose, is still divisible into many individual rickettsiae. It is assumed that an ID may be handled as a mathematical unit for statistical purposes, as for instance in calculating thermal death-time end points.

Results

The results obtained in the comparative testing of four methods of detecting *C. burnetii* in milk are listed in table 8. These tests were conducted at different temperatures, with the organism suspended in skim milk in log dilutions. The holding time was kept constant at 30 minutes in an effort to explore the relationship between the methods used in our study and the methods used in the commercial pasteurization of milk. Although the different numbered tests were conducted at different times, the four methods under study were used at the same time in each test.

Twenty-five comparative tests were made. In each test we attempted to ascertain the end point of infectivity by each method in both the

Table 8. Comparison of results of four methods of detecting small numbers of *Coxiella burnetii* in milk

Test No.	Temperature (° F.)	Highest positive dilution [1]							
		Unheated sample				Heated sample			
		GP1	GP2	GP1E3	E3	GP1	GP2	GP1E3	E3
1	139.5	9	≥9	≥9	≥12	8	≥8	≥8	≥9
2	143.1	8	9	9	10	<8	<9	10	
3	144.5	9	10	10	9	<8	<8	<8	8
4	146.4	9	9	9	10	<6	<6	<7	<7
5	148.0	10	10	10	<10	<6	<6	<6	<7
6	146.0	9	9	9	8	<3	<3	<3	<3
7	146.0	10	10	≥10	≥9	3	<3	<4	<3
8	147.9	9	9		≥8	<3	<3		
9	145.2	10	10	10	9	<3	<3	<3	<3
10	145.0	9	10	10	9	<3	<3	<3	<3
11	145.0	9	10	10	10	<3	<3	<3	3
12	145.0	9	9	10	9	<3	<3	<3	<3
13	144.0	10	10	10	10	4	<3	<3	<3
14	144.0	10	10	10	10	4	3	3	3
15	144.0	10	≥11	≥11	9	3	3	3	3
16	143.0	9	9	9	10	5	5	5	5
17	143.0	10	10	10	9	5	4	≥4	4
18	143.0	9	9	9	10	5	≥6	4	4
19	142.0	10	9	9	10	6	6	6	5
20	142.0	10	10	≥10	10	≥7	6	6	6
21	142.0	9	9	≥11	10	6	5	5	6
22	141.0	≥11	9	9	10	8	7	7	7
23	141.0	9	10	10	9	8	7	7	≥7
24	140.0	9	9	9	9	8	8	8	8
25	140.0	9	9	9	10	7	7	7	8

[1] Expressed as $\log_{10}$ of the highest positive dilution.
[2] No definite end point, but is at least this value.

Table 9. Evaluation of sensitivity of four methods of detecting *Coxiella burnetii* in milk

Method	Unheated samples				Heated samples			
	a_o*	a_e**	e	X^2	a_o*	a_e**	e	X^2
GP1	2	10	12.0	0.67	9	11.5	7.0	5.78
GP2	6	14	12.0		0	2.5	7.0	
GP1	2	7.5	10.5	1.72	7	9.5	6.5	2.78
GP1E3	8	13.5	10.5		1	3.5	6.5	
GP1	6	10.5	11.5	.18	8	10.0	8.0	1.00
E3	8	12.5	11.5		4	6.0	8.0	
GP2	8	11.0	11.5	.04	2	5.5	6.5	.31
E3	9	12.0	11.5		4	7.5	6.5	
GP2	0	9.0	10.0	.20	1	6.0	6.0	.00
GP1E3	2	11.0	10.0		1	6.0	6.0	
GP1E3	10	12.0	10.5	.42	1	4.5	6.0	.75
E3	7	9.0	10.5		4	7.5	6.5	

* Actual number of times one method provided a higher infectivity titer than the other three methods.
** Actual number of times one method provided a higher infectivity titer than the other three plus one-half number of times two methods were equal insensitivity.

$X^2 = \frac{(a_o - e)^2}{e}$ $df = 1$ 95 percent level = 3.84

heated and unheated samples. In this experiment the end point of infectivity was considered to be the highest dilution capable of inducing the formation of antibody in guinea pigs or the highest dilution to show evidence of multiplication in the yolk sac of a developing embryo after three successive passages.

In table 9, the ability of each method to detect small numbers of *C. burnetii* in both the heated and the unheated samples is compared with each of the three other methods used. A simple chi-square test to determine the significance of the differences was applied. The significant results of this test are also listed in table 9. In this particular test any chi-square value of 3.84 or above indicates a significant difference between the methods compared.

Discussion

Four methods of detecting small numbers of *C. burnetii* in milk are compared in table 9. From the chi-square values presented, no significant difference in sensitivity of the methods could be demonstrated with unheated milk samples. However, when heated milk samples were used and the same test of significance was applied, the GP1 method detected rickettsiae in higher dilutions of milk more often than did the other three methods. This difference was quite significant when compared with the GP2 and the GP1E3 methods. There was no significant difference in the sensitivity of the GP2, E3, and GP1E3 methods.

The only apparent difference between the heated and unheated samples of milk is the proportion of dead to viable rickettsiae present. In the unheated sample, relatively few dead rickettsiae were present, whereas in the heated samples the proportion of dead to live *C. burnetii* was great, especially near the end point of infectivity. Since the GP1 method is the only one of the four methods that could be influenced by killed rickettsiae, and since there was no significant difference in sensitivity between the methods when dead rickettsiae were relatively few, the superior sensitivity of the GP1 method when examining heated milk samples must be attributed to the antigenic stimulus of the dead *C. burnetii*. Therefore, the simpler and more economical method of one passage in guinea pigs could not be used to determine maximum survival or minimum destruction end points after heating.

No difference in ability of the GP2, E3, and GP1E3 methods to detect small numbers of viable *C. burnetii* could be demonstrated. However, the GP2 method had certain advantages over the others, the most pertinent of which is the specificity of the antibody induced in guinea pigs. The finding of rickettsia-like

bodies in the yolk sac of embryonating eggs cannot be considered proof of the presence of *C. burnetii* since other organisms cannot be distinguished from *C. burnetii* by visual means. In addition, certain field strains of *C. burnetii* might not multiply in the developing chick embryo. Therefore, the GP2 method was the only method tested that was specific, sensitive, and accurate.

Summary

Four methods of detecting the presence of small numbers of viable *Coxiella burnetii* in milk were compared, and a chi-square test of significance was applied to the results. Both unheated and heated samples of milk containing *C. burnetii* were tested by all four methods. Analysis of the results revealed no difference in the ability of the tests to detect small numbers of organisms in unheated samples. However, with heated samples, one passage in guinea pigs proved much more sensitive than the other methods used, and this sensitivity was attributed to the ability of the method to detect antibody induced by the dead rickettsiae in the sample. The method using two passages in guinea pigs seemed to be the only method studied that was both specific and sensitive in its ability to detect small numbers of viable *C. burnetii*. Therefore, this was selected as the method of choice for the thermal-inactivation studies.

V. Laboratory Thermal-Inactivation Studies With Coxiella burnetii Suspended in Whole Raw Milk

Coxiella burnetii, the organism of Q fever, has been shown to be relatively resistant to heat (*25*) and, in whole raw milk and cream, to survive pasteurization by accepted minimum standards (*24, 27*). This section, on the thermal inactivation of *C. burnetii*, is a presentation of the results of a study in which the organism in known concentration was suspended in whole raw milk and subjected to different temperatures for various lengths of time.

Materials and Methods

The materials and methods used in this study have been described in section IV, with the exception of those detailed below.

Whole Raw Milk. On the day of the test, whole raw grade A market milk, standardized to contain approximately 3.8 percent butterfat, was obtained from the department of dairy industry, University of California, Davis, Calif.

Preparation of Samples. In each thermal-resistance test, the infectivity titer of the unheated milk samples, as well as the time of maximum survival and minimum destruction of *C. burnetii* was determined.

A series of tenfold dilutions of the stock yolk-sac suspension containing *C. burnetii* was made in whole raw milk. Ten-milliliter aliquots of the 10^{-4} dilution were placed in thin-walled glass ampules and flame-sealed. The other dilutions in this same series were tightly stoppered and placed in the refrigerator. The ampules of the 10^{-4} dilution were then heated and cooled. Immediately after heating and cooling, both the heated and unheated dilutions were treated with antibiotics and inoculated into guinea pigs.

Concentration of Rickettsiae Heated. The 10^{-4} dilution of the series was chosen to be subjected to heat because experience had determined that this dilution would contain approximately 100,000 infective guinea pig doses of *C. burnetii* in every 2 ml. The maximum concentration of *C. burnetii* demonstrated in the milk of infected cows was 10,000 infective guinea pig doses (p. 10). Although considerable time and effort were expended to ascertain this figure, it was deemed safer for several reasons to use a log higher concentration in these studies (see. III).

Heating Procedure. A known concentration of organisms was heated at given temperatures for varying lengths of time. Other details of the heating and cooling procedure are described in section IV.

Determination of Survival and Destruction End Points. As used in this study, the end point of survival (S) is the maximum time that the sample could be heated at a given temperature and still contain demonstrable viable rickettsiae; the destruction or first negative end point (X) is the minimum time a sample had to be heated at a given temperature to destroy completely all evidence of viable *C. burnetii*.

Determination and Correction of Time. A chart of mechanically recorded time and temperature was used to determine the length of the holding period at the desired temperature, time in the water bath, and time required to transfer the test ampules from the water bath to the cold-water tank. The chart was checked by a manually operated stopwatch.

The corrected time was computed by adding to the measured holding time a factor representing the contribution of the heating-up and cooling-down periods. Burton's formula,[1] based on the present pasteurization curve, was used to estimate the percentage of mortality of the rickettsiae occurring during heating and cooling (*29*); this percentage is calculated in terms of the time at the holding temperature needed to accomplish the same percentage of mortality. This time, called equivalent-time correction factor, was then added to the time at the holding temperature to estimate the total or corrected thermal-inactivation time at the holding temperature. Theoretically, this method is not correct since the present pasteurization curve does not give the instantaneous-death time of *C. burnetii*. However, it is the best method available and the resultant error is relatively small.

Controls. In addition to the controls described in section IV, the use of whole raw milk as a diluent necessitated its examination for the presence of *C. burnetii* from a natural infection. Therefore, the undiluted whole raw milk diluent was inoculated into guinea pigs. The requisite of at least one completely negative dilution to ascertain infectivity end points was an additional check on this point.

[1] Burton's formula $E = \sum \frac{\Delta t}{T_0}$

Where: Δt = time increments at a given temperature

T_0 = time equivalent for pasteurization at that given temperature

Phosphatase Test. The phosphatase tests were run according to the method developed by Sanders and Sager (*30*).

Results

In the phase of the Q fever pasteurization study reported in this section, 28 time-temperature trials were made. The results of 17 of these trials were discarded and are not included in the data, 16 because of failure definitely to establish survival and destruction end points in the heated samples and 1, conducted at a temperature of 155° F., because the mechanical difficulties of sampling at the short time intervals required introduced significant error. The results of 11 trials are reported. All meet strict requirements for accuracy and cover a temperature range from 141° F. to 151° F. and time intervals from 1.74 to 55.29 minutes.

Table 10 presents the results of these 11 trials, arranged according to temperature of the milk tested. The concentration of organisms subjected to heat is listed for each temperature. These concentrations were determined as described above. Also listed for each temperature are the maximum survival (S) and minimum destruction (X) times corrected for the equivalent time factor, together with the results of the phosphatase test for all but two trials.

Analysis of the data did not reveal evidence that differences in the initial concentrations of organisms heated were due to any factor other than the sampling variance in setting up different aliquots from a single population of rickettsiae. Therefore, no correction was made for the one log difference found in the infectivity titrations of the unheated samples.

Table 11 presents the regression statistics derived by combining the two sets of figures representing the S and X end points as a single covariance set of data. Figure 3 shows the regression lines resulting from this treatment of the data.

Since the two regression coefficients for end points S and X were found not to be significantly different, the errors of estimate were combined and the average regression coefficient used to provide a realistic and reliable estimate of the parameters of the experiment, which resulted in the following equation:

Log time = 19.78711 − 0.128103 Temperature

This equation may then be taken as representing the median thermal death time line within the temperature range of the data. The difference between the two time means is 0.10108 and, under the design of this experiment, may be considered as the best estimate of the difference between end points for maximum time of survival and minimum time of destruction. The standard error of estimate of the minimum time of destruction regression line is 0.0721 at 143° F. and 0.09827 at 161° F., respectively.

The pertinent results of calculations with the above regression formula and its modifications are presented in table 12. These are arranged according to certain selected temperatures, the time point on the median thermal death time line, and the minimum time of destruction regression line intercepted by these tempera-

Table 10. Results of heating *Coxiella burnetii* in whole milk

Temperature (° F.)	Test No.	Concentration [1] of organisms heated	End points of heated samples		Equivalent time correction factor	Corrected end points heated samples		Phosphatase test [2]	
			S [3]	N [4]		S [3]	N [4]	S [3]	N [4]
141	60	6	50. 05	55. 05	0. 24	50. 29	55. 29	5. 04	2. 88
142	49	5	40. 32	45. 32	. 34	40. 66	45. 66	4. 56	1. 44
143	45	5	30. 42	35. 42	. 45	30. 87	35. 87	0	. 00
144	44	5	15. 37	20. 37	. 44	15. 81	20. 81	6. 00	1. 20
144	55	5	20. 43	25. 43	. 40	20. 83	25. 83		
144	56	4	15. 07	20. 07	. 38	15. 45	20. 45	5. 76	2. 64
145	43	4	15. 33	20. 33	. 53	15. 86	20. 86	4. 86	1. 20
146	42	4	10. 17	12. 17	. 57	10. 74	12. 74		
150	48	4	3. 38	4. 38	. 45	3. 83	4. 83	8. 40	3. 60
151	54	5	1. 25	2. 25	. 49	1. 74	2. 74	4. 80	. 72
151	59	4	2. 25	3. 25	. 52	2. 77	3. 77	10. 80	3. 12

[1] Expressed as $\log_{10}$.
[2] Expressed in mg. ml. phenol liberated. (4 mg. per milliliter or more is considered evidence of improper pasteurization).
[3] Maximum survival time in minutes.
[4] Minimum destruction time in minutes.

Table 11. Regression statistics: Laboratory thermal-resistance studies of the organism in whole raw milk

	Sums of squares and products (x = temperature, y = log time)			Sums of squares of deviations from regression	Degrees of freedom	Mean squares	F [4]
	Sx^2	Sy^2	Sxy				
Total [1]	253. 46	4. 308435	−32. 46941	0. 148952	20		
Between [2]	0	. 056638	0	0. 056638	1	0. 056638	11.7 [5]
Within [3]	253. 46	4. 251797	−32. 46941	. 092314	19	. 0048586	
Difference between S and N regression slopes.				. 005479	1	. 005459	1.13 N. S.
Deviations from individual regressions.				. 086855	18	. 004825	

Using the average regression, −0.128105 and the summed time and temperature, y − intercept = 1.14202 + 0.128105 · 145.547 = 19.78711, regression equation is: Log time = 19.78711 − 0.128135 Temperature

[1] The "total" row represents overall variation in the entire set of last positive times (S) and first negative times (N).
[2] The "between" row represents differences in the means of the two subgroups, S and N. Thus, .056638 = $11\ 2\ (\bar{y}_S - \bar{y}_N)^2$. Since both last positive and first negative times were obtained at the same temperature, the terms Sx^2 and Sxy are zero in this row.
[3] The "within" row represents the sum of the separate variations within each subgroup.
[4] Ratio of mean squares.
[5] Highly significant ($P < .01$).

tures. In addition, the intercept of the minimum time of destruction regression line plus a 2σ interval is also listed for each temperature. The 2σ interval represents a 97.7 percent confidence level as a margin of safety.

Discussion

In the laboratory study of thermal inactivation of *C. burnetii*, well-controlled experimentation could be conducted in the temperature range 141° F. to 151° F. The absence of good sampling devices permitting accurate observations in the temperature range 155° F. to 163° F., together with the extremely short time intervals necessary, prevented controlled observations in this temperature zone.

Although certain statistical considerations limit the reliability of extrapolation into the higher temperature range, extrapolation was done as the only method of indicating the time-temperature relationships to be found there. The statistical treatment of the data, the 2σ or 97.7 percent confidence interval added to the minimum time of destruction regression line, and the use of a relatively large population of organisms all tend toward minimizing the dangers of this extrapolation. Nevertheless, the effect of the time scale on the data of this experiment should be borne in mind.

Figure 3. Results of laboratory thermal-resistance studies of *Coxiella burnetii* in whole raw milk.

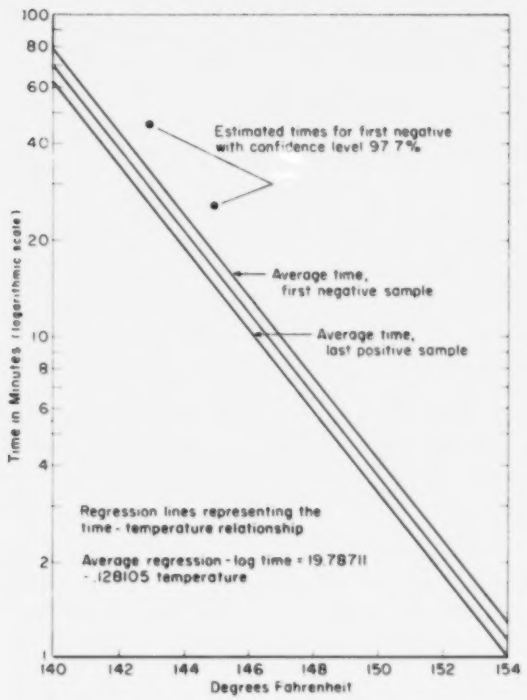


Table 12. Times at pertinent temperatures on the regression lines derived from regression statistics

Temperature (° F.)	Regression lines		
	Median death time end point	Minimum time of destruction	Minimum time of destruction plus 2σ
	Minutes	*Minutes*	*Minutes*
143	29. 39	33. 02	46. 03
145	16. 29	18. 31	25. 42
	Seconds	*Seconds*	*Seconds*
160	11. 7	13. 2	20. 4
161	8. 7	9. 8	15. 4
162	6. 5	7. 3	11. 6

The log interval for the time interval 50 to 55 minutes is 0.041, for 25 to 30 minutes, 0.079, and for 2 to 3 minutes, 0.177. These differences in scaling are reflected as a shift in the slope of the regression between maximum time of survival and minimum time of destruction. The true slope is intermediate although it could possibly reach either value. The data show no significant difference in the two regressions over the temperature range sampled. However, considerable difference results when extrapolations are made into the higher temperature range. The statistical treatment summarized in table 11 reduces this difference to as minimal a value as the conditions of the experiment permit. Therefore, the resulting regression lines may be accepted as the best available estimate of the thermal death-time of *C. burnetii* in whole raw milk.

From the data listed in table 12 it is evident that heating whole raw milk for 30 minutes at 143° F. is inadequate to destroy all rickettsiae, whereas heating for 30 minutes at 145° F. results in complete destruction of the organisms plus a 2σ confidence interval in less than 30 minutes. Extrapolation of the test results indicates that a comparable relationship may

exist when milk is heated at 160° F. and at 161° F. for 15 seconds.

Summary

Coxiella burnetii, the organism of Q fever, is likely to be found in the milk of infected dairy cows. Results of a laboratory thermal-inactivation study of *C. burnetii* showed that heating whole raw milk containing concentrations of the organism for 30 minutes at 143° F. is inadequate to destroy all organisms, whereas heating the milk at 145° F. for 25.42 minutes results in their complete destruction, with a 97.7 percent level of confidence. Projection of the data from the experimental temperature range of 141° F. to 151° F. to higher temperature ranges indicates that heating at 160° F. for 15 seconds may also be inadequate to eliminate all viable *C. burnetii* in these concentrations from whole raw milk; however, heating the milk at 161° F. for 15.4 seconds would result in destruction of the organisms with a 97.7 percent level of confidence.

VI. Application of Laboratory Study Findings to Commercial Pasteurization Equipment

Since the over-all objective of the thermal-inactivation study was to ascertain the minimum procedures that will eliminate viable *Coxiella burnetii* in pasteurizing milk on a commercial scale, an attempt was made to relate the findings of the laboratory study to commercial processes.

It was realized from the beginning that studies involving commercial equipment presented certain problems. One of the more important of these was the variation that might be expected in the heating and cooling characteristics of commercial pasteurizers, especially in the high-temperature short-time (HTST) units. Variation occurs in the heat-up and cool-down rates of HTST equipment produced by different manufacturers and also in these rates in the same unit when it is operated at different capacities. It was obviously impossible to study all such units. However, the fundamentals of thermal inactivation of micro-organisms—heat-up, holding and cool-down, and temperature control—are common to all pasteurization methods. Pasteurization equipment was selected which was representative of that commonly employed commercially.

This section presents the results of a study in which a known concentration of *C. burnetii* was suspended in whole raw milk and subjected to heat in one type of vat pasteurizer and in one type of HTST pasteurizer. The equipment and controls were designed and operated in conformance with the specifications contained in the Milk Ordinance and Code recommended by the Public Health Service (*29*).

Materials and Methods

The methods outlined in this section were the same as those described in section V, with the following exceptions.

Whole Raw Milk. On the day before the test run, 100 gallons retail grade A whole raw milk whose butterfat content had been standardized to approximately 3.8 percent was obtained from the creamery. The milk was received in a cold-wall tank, where it was kept at a temperature of 36° to 40° F. A sample of this milk was inoculated into guinea pigs, using the GP1 method, as a control against milk containing *C. burnetii* from a naturally infected cow.

Concentration of C. burnetii. On the morning of the test, a culture of *C. burnetii* was added to the cold milk. The rickettsiae were contained in a yolk sac suspension prepared as described in section V. Enough of the organisms were added to insure a final dilution

approximating 100,000 infective guinea pig doses per milliliter, as explained in section V. The suspension of organisms and milk was stirred vigorously by mechanical means for 30 minutes, after which a sample was obtained and the infectivity titer was determined in guinea pigs by the GP2 method.

Movement of Milk. The milk was moved from the cold-wall receiving tank by means of a conventional sanitary milk pump, conducted through stainless steel tubing and couplings constructed in conformance with 3-A sanitary standards (*41*), and delivered either directly to the vat pasteurizer or to the float tank of the HTST equipment.

Vat-Type Pasteurization. A conventional 100-gallon vat equipped with an agitator and an airspace (foam) heater was used for vat pasteurization. The milk in the vat was heated by steam-heated water circulating in an outer jacket. The temperature of the water was controlled thermostatically. The temperature of the milk was continuously inscribed on a standard recording thermometer that had been checked for accuracy. The temperature of the airspace above the milk was kept 5 degrees above the temperature of the milk at all times, except during trial 62 (table 13). In addition, thermocouples were placed in various parts of the vat on several occasions to determine that the milk and the airspace above it were being evenly heated. The milk was brought to the temperature being investigated in approximately 30 minutes, at which time samples were quickly withdrawn every 5 minutes by means of sterile syringes and injected into sterile containers kept frozen in ice. Cooling of the sample to below 140° F. was practically instantaneous.

The vat was emptied and sterilized by filling it with hot water, which was maintained at a temperature of 180° F. for 30 minutes and then cooled. After cooling, the water was tested for the presence of *C. burnetii* on several occasions and always was found negative. The vat was scrubbed with commercial detergents and rinsed thoroughly before being used again.

HTST Pasteurization. A conventional type unit designed to operate at a capacity of from 1,000 to 2,000 lbs. of milk per hour was used for HTST pasteurization. The unit consisted of a float tank; a press consisting of a heating, a regenerating, and a cooling unit; a holding tube and a flow-diversion valve; a complete control panel; a hot water heating unit operated by steam; and a sweet water (chilled water) cooler. The compressors and sweet water cooler were installed in adjacent rooms. Sampling ports were installed at the entrance to and at the end of the holding tube. The sampling ports presented no impeding surface to the milk inside of the holding tube.

The temperature of the milk was obtained at the flow-diversion valve by standard indicating and recording thermometers, which were checked for accuracy. From time to time, the temperatures of the milk were ascertained at this point, at the entrance to the holding tube, and in different parts of the press by means of thermocouples, and were recorded.

The speed of flow of the milk through the holding tube was obtained by the method described in appendix H of the Milk Ordinance and Code—1953 recommendations of the Public Health Service (*29*). This factor was determined each time the equipment was used and, if necessary, the milk pump was reset.

In the experimental tests, three temperatures were investigated during the time it took for 100 gallons of milk to run through the pasteurizer. The temperatures were spaced at 2-degree intervals; the highest temperature was investigated first and the lowest, last. To prevent the double exposure of some milk to the heating process, the flow-diversion valve was adjusted to divert the flow of milk just below the lowest temperature. Therefore, the valve did not operate during a test run. Nevertheless, a record of the temperatures was obtained at the flow-diversion valve control-bulb position, and close inspection of these records proved that the test temperatures were being maintained. At each adjustment of temperature, enough milk was allowed to run through the machine to insure none of it being heated above that temperature.

In each experimental run at each temperature, samples of milk were taken at both sampling ports and at the end of the cooling section. These samples were withdrawn directly into containers kept in ice and were chilled below

140° F. almost instantaneously. The samples of milk were injected into guinea pigs on the same day and the presence or absence of viable *C. burnetii* was determined by the GP2 method. Phosphatase tests were run on the majority of samples.

The rate of flow of the milk emerging from the cooler section was determined for each test. After each test, water heated to 180° F. in the heater section was run through the pasteurizer in large amounts. On occasion, this water was tested for the presence of *C. burnetii* but always was found negative. After sterilization with hot water, the machine was dismantled and scrubbed with detergents.

Safeguards. All milk used in the test runs was discharged into a second vat-type pasteurizer, where it was heated to 180° F. and held at this temperature for 1 hour before disposal. The milk was never found to contain viable *C. burnetii* after this treatment.

The pasteurization room was well ventilated, with a calculated 8 to 10 complete changes of air every hour.

All personnel on the project were inoculated with a formol-killed suspension of *C. burnetii* before being allowed to enter a hazardous area. The regime of inoculations was such that demonstrable complement-fixing antibody to the rickettsiae was induced. No personnel contracted Q fever.

Results

Vat-Type Pasteurization Equipment. Table 13 summarizes data from the eight pasteurization trials using vat-type equipment. The data are listed according to the holding temperature of the trials. The concentration of organisms in the raw milk before heating, the maximum survival end point (S), the minimum destruction end point (N), and the time to reduce the phosphatase to a level of 4 mg. of phenol per milliliter are given for each trial. In addition, the results of testing the raw milk for the presence of *C. burnetii* from natural sources are given. This raw milk was used later to suspend the rickettsiae.

In trial No. 62 the airspace heater did not function properly and this probably accounts for our inability to demonstrate end points in the heated samples. For this reason, the data obtained in trial No. 62 were not plotted in figure 4. However, suitable end points were determined with the heated samples in each of the other seven trials.

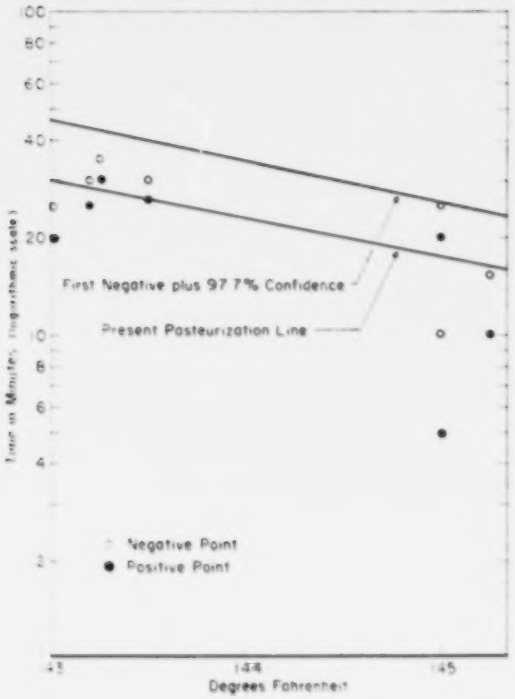
The concentration of organisms in the milk before heating was not determined in three trials, Nos. 63, 65, and 72. In trials Nos. 63 and 65, the raw milk used in the test contained *C. burnetii* from natural sources. Since this same raw milk was used as a diluent to determine the infectivity titer of the unheated milk, the concentration of organisms to be heated could not be determined because the infectivity end point was masked. However, the maximum survival end point (S) and the minimum destruction end point (N) in trials Nos. 63 and 65 were comparable to those in other trials with known concentrations of organisms in the unheated milk. For this

Table 13. Results of pasteurization of milk with vat-type equipment

Trial No.	Temperature (° F.)	Concentration of organisms heated [1]	Raw milk control	Maximum survival end point (S) (minutes)	Minimum destruction end point (N) (minutes)	Phosphatase test [2]
62	143.0	5		[3] 40		26.63
63	143.2	[3] 6	+	25	30	27.49
67	143.0	5		20	25	31.26
68	143.2	6		30	35	27.65
73	143.5	5		26	30	26.51
65	145.0	[3] 6	+	5	10	16.55
66	145.2	5		10	15	15.44
72	145.0	[3] 8	Ac.[4]	20	25	18.13

[1] Expressed as $\log_{10}$.
[2] Reduction time in minutes to a level of 4 mg. of phenol per milliliter.
[3] End point not determined, but is at least this value.
[4] Anticomplementary activity of serums.

Figure 4. Results of trials of vat-type equipment in pasteurization of whole raw milk.



reason it may be assumed that the effect of the natural contamination was negligible.

In trial No. 72, in which the concentration of organisms to be heated was not determined because of the anticomplementary activity of the serums, the diluent used to obtain infectivity end points was sterile skim milk and, therefore, no masking effect was involved. For this reason, it must be assumed that the concentration of organisms heated in this trial was in excess of the number found in 100 million infective guinea pig doses or greater than 1,000 times the number subjected to heat in trials Nos. 63 and 65. The data obtained in trial No. 72 are plotted in figure 4.

In the other five trials the concentration of organisms heated, the maximum survival time, and the minimum destruction time were determined and are listed in table 13.

Figure 4 shows the present pasteurization curve and the regression line of the minimum destruction end points (X) with a 2σ confidence interval from figure 3. Also plotted in figure 4 are the maximum survival and the minimum destruction end points obtained with the vat-type pasteurization equipment.

HTST Pasteurization Equipment. The pertinent data derived from observations using the HTST type of pasteurization are presented in table 14. In the 5 trials listed, 15 temperature points were studied. For each temperature the time for heating to the holding temperature, the time in the holding tube, and the time from the holding tube to the exit from the cooling section of the press are listed, together with the total time for each point. The time increments are expressed in equivalents at the holding temperature.

The heating-up time was determined from the heat-up curve furnished by the manufacturer and by estimating flow times and temperatures. These estimations were made by inserting thermocouples into the heating and regenerating sections of the press, and by inserting thermocouples and electrodes of the solubridge salt timer into the vat at the sampling ports. Shown also are the phosphatase determinations on samples collected at each port and the results of testing these samples for the presence of viable *C. burnetii*.

The concentration of organisms heated in each trial, also listed in table 14, was somewhat above the 100,000 infective guinea pig doses desired. Due to the length of time required for determination of this error in dilution of the 100 gallons of milk to be used in the test, the error was not discovered until the study was completed. However, the larger population of *C. burnetii* subjected to heat may be considered as an additional safety factor in the experiment.

The present pasteurization curve is shown in figure 5, together with the results obtained at all 3 sampling ports for the 15 temperatures studied. Inasmuch as the timing pump was run at approximately the same speed for all trials, the sampling times for each station are relatively uniform throughout the experiment.

Discussion

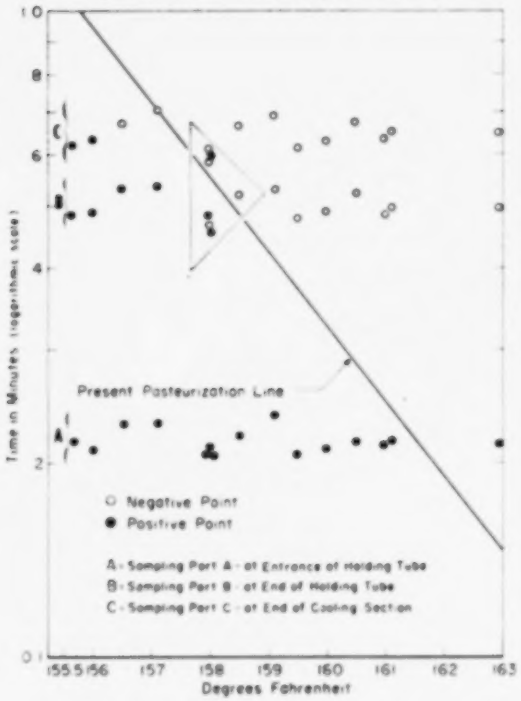
The results of milk pasteurization with the vat-type pasteurizer are plotted in figure 4. It is readily discernible that the present pasteurization standards in the 143° F. to 145° F. temperature range are inadequate to destroy *C. burnetii* when the organisms are suspended

Table 14. Results of pasteurization of milk with high-temperature short-time equipment

Trial No.	Concentration heated [1]	Temperature (° F.)	Method [2]	Heating time to A [3] (min.)	Phosphatase test	Q	Time A to B [3] (min.)	Heating time to B (min.)	Phosphatase test	Q	Heating time B to C [3] (min.)	Time to C [3] (min.)	Phosphatase test	Q
		163	Calc	0.185	5.04	[4] −	0.279	0.464	0.48	[4] −	0.148	0.612	0.00	[4] −
			HC	.215			.279	.494			.153	.647		
69	−7	161	Calc	.168	35.0	[4] +	.270	.438	.48	−	.148	.586	.48	−
			HC	.215			.270	.485			.150	.635		
		158	Calc	.165	60.7	+	.259	.424	1.90	−	.204	.628	.72	−
			HC	.207			.259	.466			.122	.588		
		159.5	Calc	.168		+	.269	.437		−	.125	.562		−
			HC	.208			.269	.477			.139	.616		
70	6	158.0	Calc	.169			.271	.440		[4] +	.125	.565		−
			HC	.211			.271	.482			.132	.614		
		155.7	Calc	.179		+	.270	.449		+	.129	.578		[4] +
			HC	.217			.270	.487			.131	.618		
		160.0	Calc	.153	72.0	+	.280	.433	1.69	−	.146	.579	.72	−
			HC	.211			.280	.491			.140	.631		
71	6	158.0	Calc	.160	169.9	+	.253	.413	5.25	+	.143	.556	.72	+
			HC	.207			.253	.460			.144	.604		
		156.0	Calc	.141	286.1	+	.282	.423	50.4	+	.144	.567	17.3	+
			HC	.209			.282	.491			.140	.631		
		160.5	Calc	.195	49.4	+	.306	.501	1.2	−	.175	.676	.24	−
			HC	.216			.306	.522			.151	.673		
74	7	158.5	Calc	.178	288.0	+	.299	.477	2.4	−	.178	.655	.48	−
			HC	.221			.299	.520			.143	.663		
		156.5	Calc	.190	549.1	+	.297	.487	19.9	+	.182	.669	4.04	−
			HC	.230			.297	.527			.144	.671		
		161.2	Calc	.176	37.9	+	.283	.459	.00	−	.148	.607	.00	−
			HC	.216			.283	.499			.153	.652		
75	6	159.2	Calc	.194	99.6	+	.292	.486	.72	−	.198	.684	.00	−
			HC	.237			.292	.529			.162	.691		
		157.1	Calc	.222	292.8	+	.306	.528	10.56	+	.160	.688	2.4	−
			HC	.230			.306	.536			.166	.702		

[1] Expressed as $\log_{10}$.
[2] Calc.—equivalent time in minutes derived by inserting thermocouples and electrodes into the equipment.
HC—equivalent times in minutes derived from curve supplied by the manufacturer.
[3] A, entrance to holding tube; B, end of holding tube; C, end of cooling section.
[4] +, viable *C. burnetii* present; −, *C. burnetii* not demonstrated.

Figure 5. Results of high-temperature short-time pasteurization of whole raw milk.


Time in Minutes (logarithmic scale)
Present Pasteurization Line
○ Negative Point
● Positive Point
A - Sampling Port A - at Entrance of Holding Tube
B - Sampling Port B - at End of Holding Tube
C - Sampling Port C - at End of Cooling Section
Degrees Fahrenheit

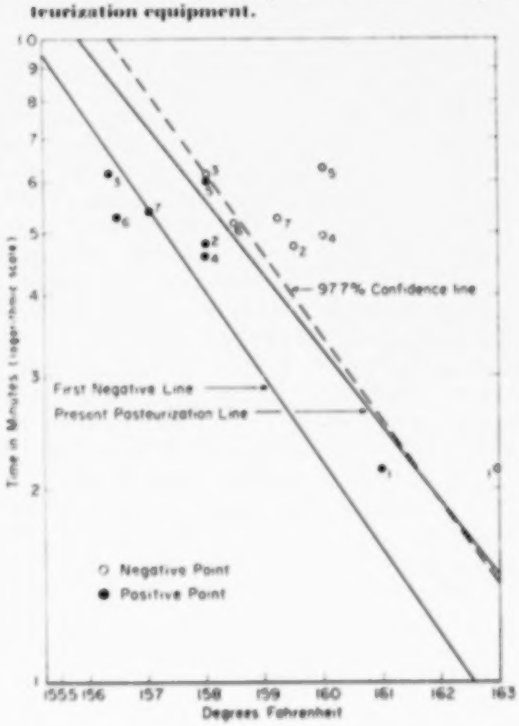
in whole milk. In each trial the minimum destruction end point (X) determined in this type of pasteurization equipment was below the minimum destruction plus a 2σ confidence level regression line derived from the statistical treatment of the data determined in the laboratory study. The point 145° F. for 30 minutes is well above the laboratory determined regression line and the points obtained with the vat pasteurizer. This is so even in trial No. 72 in which more than 100 million infective guinea pig doses of *C. burnetii* were subjected to heat.

The data obtained with the HTST pasteurization equipment are plotted in figures 5 and 6 and show the relationship of these findings to the present pasteurization curve and the line obtained by extrapolation of the minimum destruction end point plus the 2σ confidence level data obtained in the laboratory thermal-inactivation study. The use of a 15-second holding time and a 2-degree temperature interval does not allow the precise estimation of either the maximum survival or the minimum destruction end points without too great an error of estimate.

The 45 survival and destruction points obtained by using the HTST equipment are plotted in figure 5. The time increments are the equivalent times (HC') derived from the curve supplied by the manufacturer. In only one instance did a maximum survival point fall above the present pasteurization line. Had the time increment calculated from the flow measurements been used, this point would have fallen on the regression line. Furthermore, three minimum destruction points are located below this line and all other survival points fall well below the line derived from the projection of the laboratory data.

In figure 6, seven pairs of survival and destruction points obtained with the HTST pasteurization unit are plotted in relation to the present pasteurization curve and the minimum survival curve plus the 2σ confidence level line extrapolated from the laboratory data. Each pair of points consists of a maxi-

Figure 6. Paired positive and negative points obtained with high-temperature short-time pasteurization equipment.



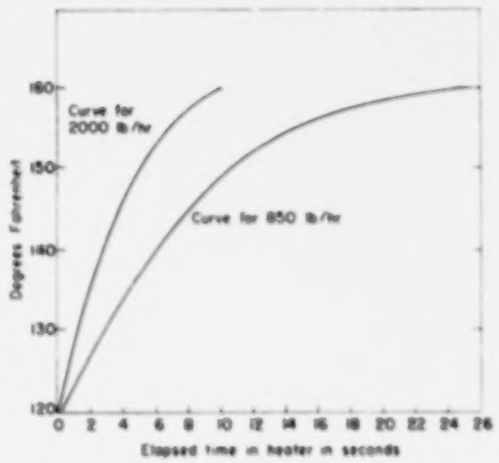
mum survival and a minimum destruction point obtained from the same sampling port during the same run by lowering the temperature of the run by 2 degrees while keeping the time constant. The corresponding points of each pair are designated by the same number. Examination of the location of these paired points substantiates the related finding in figure 5 that the present pasteurization curve lies very close to the thermal-inactivation end point of *C. burnetii* suspended in whole raw milk. From figure 6 it is also evident that all positive points obtained in this study lie below the line extrapolated from the laboratory data.

Consideration of the survival and destruction points located within the triangle in figure 5 further strengthens the impression that the present pasteurization line approximates the thermal inactivation of *C. burnetii*. Here there are positive and negative points within a 0.5° F. and 0.12-minute range. This relatively narrow range straddles the pasteurization curve but is well below the line derived by extrapolating the laboratory data. Although there is still no information on the precise thermal-inactivation end point of *C. burnetii* to be derived from these observations, the presentations in figures 5 and 6 lend confidence to the extrapolation of the regression line obtained in the lower temperature ranges.

As pointed out earlier in this section, the observations made while using the HTST pasteurization equipment include the total heat treatment heat-up, holding, and cool-down of the organism. Because of variations in equipment design and rates of product flow, pasteurization was based on maintaining a time-temperature combination adequate for the destruction of micro-organisms in the holding tube; therefore, the data obtained in the commercial equipment studies must be interpreted in relation to these facts. The HTST press used in these experiments was engineered for a maximum capacity of 2,000 lbs. per hour but was operated at only 850 lbs. per hour.

Figure 7 presents the heat-up curve as furnished by the manufacturer for this press at both 850 and 2,000 lbs. per hour. When Burton's formula (*39*) is applied to these

Figure 7. Heating curves of high-temperature short-time pasteurization equipment at pressures of 2,000 lbs. and 850 lbs. per hour.



curves, it is found that before entering the holding tube the organisms have been subjected to a pressure of 2,000 lbs. for 0.056 minutes, or 3.36 seconds, and to a pressure of 850 lbs. for 0.151 minutes, or 9.06 seconds, at 161° F. If the pasteurizer is operated at the accepted holding time of 15 seconds, the total times from the entrance of the holding tube to the diversion valve at 2,000 lbs. and at 850 lbs. pressure are 18.36 and 24.06 seconds, respectively. As the milk is cooled, there is also a slight continued heat effect. Therefore, if the 15-second interval in the holding tube is strictly maintained, and if the thermal-regression curve extrapolated from the laboratory data and possessing a 97.7 confidence level above the minimum time of destruction end point which intercepts 161° F. at 15.4 seconds is accepted, it is highly improbable that the total heat treatment can fall below this time-temperature combination. This assumption is further supported by studies of the commercial equipment. In these studies, when milk was processed at temperatures above 158° F., no viable *C. burnetii* were recovered from samples collected at the discharge port of the holding tube.

The number of safety factors included in this experiment, together with the use of concentrations of organisms 10 to 10,000 times the number demonstrated in the milk of infected cows, still further reduces the prob-

ability of *C. burnetii* surviving HTST pasteurization at the minimum recommended standards when suspended in standardized whole raw milk.

Summary

Results obtained with commercial equipment in pasteurizing whole milk containing *Coxiella burnetii*, the organism of Q fever, are presented.

Results of pasteurization with vat-type equipment support the findings of the laboratory thermal-inactivation study that a temperature of 143° F. for 30 minutes is inadequate to eliminate viable *C. burnetii* from milk, whereas heating the milk for the same time interval at 145° F. insures the elimination of these organisms with a high level of confidence.

Observation of the results of using high-temperature short-time pasteurization equipment tend to confirm the extrapolated regression line derived from the laboratory data and strongly support the presently recommended standard of pasteurization at 161° F. for 15 seconds as adequate to eliminate viable *C. burnetii* from whole milk.

VII. Recommendations and Conclusions

The agreement between the University of California and the Public Health Service implementing the milk survey stipulated as one of its objectives possible modifications, if indicated, of the time and temperature combinations used in commercial pasteurization processes. A study group was convened to consider the project data from this viewpoint. In addition to the authors, the group included Ernest B. Kellogg, J. R. Meyers, and C. B. Christiansen, representing the Milk Industry Foundation; and Roberts Everett, George Putnam, and Horace Mitten, Jr., representing the Dairy Industries Supply Association, Inc. Representing the Public Health Service were Dr. James H. Steele, Dr. L. Otis Emik, and Dr. R. A. Tjalma, of the Communicable Disease Center; Dr. Keith H. Lewis, Dr. L. A. Black, and Dr. Eugene K. Harris, of the Robert A. Taft Sanitary Engineering Center; and John D. Faulkner and Harold B. Robinson, of the Milk and Food Program, Division of Sanitary Engineering Services.

It was the consensus of the study group that the project data indicated clear need to increase the temperature of the milk in vat pasteurization. The group further agreed that pasteurization of milk at 145° F. for 30 minutes by the vat method, or at 161° F. for 15 seconds by the high-temperature short-time method was adequate for the protection of the public health. However, no data were available relative to the effectiveness of these temperatures for the pasteurization of other dairy products. Since it is recognized that temperatures slightly higher than 161° F. are required to destroy some micro-organisms when they are suspended in products whose fat content is higher than that of milk or which contain added sugar, it was recommended that a somewhat higher temperature be used for the pasteurization of these products.

It was also the consensus of the group that this recommendation should be disseminated to State and local milk control authorities and to the dairy industry. Therefore, the Public Health Service prepared a memorandum to all State and Territorial milk control authorities and others concerned (see p. 28).

DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
WASHINGTON 25, D. C.

BUREAU OF STATE SERVICES

July 16, 1956

TO: State and Territorial Milk Control Authorities
and All Others Concerned

SUBJECT: Effectiveness of Pasteurization for the Destruction of the Causative Agent of Q Fever in Milk

Epidemiological investigations have revealed that cows, sheep, and goats, when infected with Coxiella burnetii, are important sources of Q fever infection in man, and that the organism is shed in the milk as well as other secretions and excretions of such animals. Although most studies of the occurrence of Q fever point to the inhalation of dust contaminated by excretions of infected animals as the most common source of human infection, studies in endemic areas, where dairy cattle represent the primary source, show that the incidence of Q fever infection is much higher in persons using raw milk than in those using pasteurized milk.

A research project was undertaken to determine pasteurization times and temperatures necessary to assure destruction of this organism in milk. This project, a cooperative undertaking of the University of California, the Milk Industry Foundation, the Dairy Industries Supply Association, and the Public Health Service, has shown that when large numbers of the organism are present, some may survive pasteurization at 143° F. for 30 minutes. However, the findings indicate that pasteurization of milk at 145° F. for 30 minutes by the vat method, or at 161° F. for 15 seconds by the high-temperature short-time method, is adequate to protect the public health.

Investigations with dairy products other than milk and skim milk have not been sufficient to establish whether the above times and temperatures are adequate for the destruction of C. burnetii in products containing higher fat or added sugar such as half-and-half, cream, and flavored milk and skim milk beverages. It is recognized that higher temperatures are required to destroy some microorganisms when suspended in such products, and it is suggested that at least an additional 5° F. of temperature be employed for the pasteurization of milk products containing higher fat or added sugar. A recent survey of approximately 500 plants indicates that temperatures of 150° F. for the 30-minute vat method, and 166° F. for the H.T.S.T. method, or higher temperatures, are now being employed by most plants in the commercial pasteurization of such products. Therefore, the increase of an additional 5° F. should present no problem to the dairy industry.

It is suggested that this information be furnished to all milk sanitation authorities within your State. The Milk Industry Foundation is forwarding copies of this memorandum to its membership.

Ole L. Anderson

Assistant Surgeon General
Chief, Bureau of State Services

References

(1) Derrick, E. H.: "Q" fever, A new fever entity: Clinical features, diagnosis, and laboratory investigation. M. J. Australia 2:281-299 (1937).

(2) Berge, T. O., and Lennette, E. H.: World distribution of Q fever: Human, animal and arthropod infection. Am. J. Hyg. 57:125-145, March 1953.

(3) Wentworth, B. B.: Historical review of the literature on Q fever. Bact. Rev. 19:129-148, September 1955.

(4) Topping, N. H., Shepard, C. C., and Irons, J. V.: Q fever in the United States: I. Epidemiological studies of an outbreak among slaughterhouse workers. J. A. M. A. 33:813-815 (1947).

(5) Enright, J. B.: The role of animals in Q fever. Vet. Med. 46:383-386, 399, October 1951.

(6) Beck, M. D., Bell, J. A., Shaw, E. W., and Huebner, R. J.: Q fever studies in southern California: II. An epidemiological study of 300 cases. Pub. Health Rep. 64:41-56, Jan. 14, 1949.

(7) Lennette, E. H., Dean, B. H., Abinanti, F. R., Clark, W. H., Winn, J. F., and Holmes, M. A.: Q fever in California: V. Serologic survey of sheep, goats and cattle in three epidemiologic categories, from several geographic areas. Am. J. Hyg. 54:1-14, July 1951.

(8) Lennette, E. H., Clark, W. H., and Dean, B. H.: Sheep and goats in the epidemiology of Q fever in southern California. Am. J. Trop. Med. 29:427-541, July 1949.

(9) Bell, J. A., Beck, D. M., and Huebner, R. J.: Epidemiological studies of Q fever in southern California. J. A. M. A. 142:868-872 (1950).

(10) Davis, G. E., Cox, H. R., Parker, R. R., and Dyer, R. E.: A filter-passing infectious agent isolated from ticks: I. Isolation from *Dermacentor andersoni*, reactions in animals, and filtration experiments. Pub. Health Rep. 51:2259-2267, Dec. 20, 1936.

(11) Jellison, W. L., Bell, E. J., Huebner, R. J., Parker, R. R., and Webb, H. H.: Q fever studies in southern California: IV. Occurrence of *Coxiella burnetii* in the spinose ear tick, *Otobius megnini*. Pub. Health Rep. 63:1483-1489, Nov. 12, 1948.

(12) Parker, R. R., and Stewman, G.: Spontaneous infection of the brown dog tick, *Rhipicephalus sanguineus* with *Coxiella burnetii*. Pub. Health Rep. 61:1159-1160, Sept. 9, 1949.

(13) Huebner, R. J., Jellison, W. L., Beck, M. D., Parker, R. R., and Shepard, C. C.: Q fever studies in southern California: I. Recovery of *Rickettsia burneti* from raw milk. Pub. Health Rep. 63:214-222, Feb. 13, 1948.

(14) Bell, E. J., Parker, R. R., and Stewmon, H. G.: Q fever, experimental Q fever in cattle. Am. J. Pub. Health 39:478-484, Apr. 1949.

(15) Jellison, W. L., Webb, H. H., Ehrs, R. E., and Huebner, R. J.: Q fever studies in southern California: XI. Recovery of *Coxiella burnetii* from milk of sheep. Pub. Health Rep. 65:395-399, Mar. 24, 1950.

(16) Winn, J. F., Lennette, E. H., Webb, H. H., and Abinanti, F. R.: Q fever studies: XVII. Presence of *Coxiella burnetii* in the feces of sheep. Am. J. Hyg. 59:183-189, Sept. 1953.

(17) Abinanti, F. R., Lennette, E. H., Winn, J. F., and Webb, H. H.: Q fever studies: XVIII. Presence of *Coxiella burnetii* in the birth fluids of naturally infected sheep. Am. J. Hyg. 58:295-298, November 1953.

(18) Webb, H. H., Lennette, E. H., Abinanti, F. R., and Winn, J. F.: Q fever in California: IV. Occurrence of *Coxiella burnetii* in the placenta of naturally infected sheep. Pub. Health Rep. 66:1473-1477, Nov. 9, 1951.

(19) Jellison, W. L., Ormsbee, R., Beck, M. D., Huebner, R. J., Parker, R. R., and Bell, E. J.: Q fever studies in southern California: V. Natural infection in a dairy cow. Pub. Health Rep. 63:1641-1648, Dec. 10, 1948.

(20) Lenette, L., and Huebner, R. J.: Q fever studies in southern California: IX. Isolation of Q fever organism from parturient placentas of naturally infected dairy cows. Pub. Health Rep. 65:537-544, Apr. 21, 1950.

(21) Delay, P. D., Lennette, E. H., and Deibner, K. R.: Q fever in California: II. Recovery of *Coxiella burnetii* from naturally-infected air-borne dust. J. Immunol. 65:211-220, August 1950.

(22) Sigel, M. M., Scott, T. F. McN., and Heuke, W.: Q fever in a wool and hair processing plant. Am. J. Pub. Health 40:524-532, May 1950.

(23) Abinanti, F. R., Webb, H. H., Winn, J. F., and Lennette, E. H.: Q fever studies: XIX. Presence and epidemiologic significance of *Coxiella burnetii* in sheep's wool. Am. J. Hyg. 61:362-370, May 1955.

(24) Huebner, R. J., Jellison, W. L., Beck, M. D. and Wilcox, F. P.: Q fever studies in southern California: III. Effects of pasteurization on survival of *C. burnetii* in naturally infected milk. Pub. Health Rep. 64:499-511, Apr. 22, 1949.

(25) Ransom, S. E., and Huebner, R. J.: Studies on the resistance of *Coxiella burnetii* to physical and chemical agents. Am. J. Hyg. 53:140-149, January 1951.

(26) Marmion, B. P., MacCallum, F. O., Rowlands, A., and Thiel, C. C.: The effect of pasteurization on milk containing *Rickettsia burnetii*. Monthly Bull. Ministry of Health and the Public Health Lab. Service (England) N. I. H. D. Nos. 1175 10:119-128, May 1951.

(47) Lennette, E. H., Clark, W. H., Abinanti, M. A., Brunetti, O., and Covert, J. M.: Q fever studies: XIII. The effect of pasteurization on *Coxiella burneti* in naturally infected milk. Am. J. Hyg. 55:246–253, March 1952.

(48) Jellison, W. L., Huebner, R. J., Beck, M. D., Parker, R. R., and Bell, E. J.: Q fever studies in southern California: VIII. Recovery of *Coxiella burneti* from butter made from naturally infected and unpasteurized milk. Pub. Health Rep. 63:1712–1714, Dec. 31, 1948.

(49) U. S. Public Health Service: Milk Ordinance and Code 1953 recommendations of the Public Health Service. PHS Pub. No. 229. Washington, D. C., U. S. Government Printing Office, 1956.

(50) Clark, W. H., Lennette, E. H., and Romer, M. S.: Q fever in California: XI. An epidemiologic summary of 350 cases occurring in northern California during 1948–1949. Am. J. Hyg. 54:319–330, November 1951.

(51) Clark, W. H., Romer, M. S., Holmes, M. A., Welsh, H. H., Lennette, E. H., and Abinanti, F. R.: Q fever in California: VIII. An epidemic of Q fever in a small rural community in northern California. Am. J. Hyg. 54:25, July 1951.

(52) Lennette, E. H., and Clark, W. H.: Observations on the epidemiology of Q fever in northern California. J. A. M. A. 145:308 (1951).

(53) Shepard, C. C.: Q fever: A serological survey of bovine serums in the United States. Am. J. Trop. Med. 28:948, November 1948.

(54) Lusto, L., Huebner, R. J., and Stoenner, H. G.: Q fever studies in southern California: XII. Aureomycin treatment of dairy cattle naturally infected with *Coxiella burneti*. Pub. Health Rep. 66:199–204, Feb. 16, 1951.

(55) Lusto, L., and Mason, D. M.: An agglutination test for bovine Q fever performed on milk samples. J. Immunol. 74:222–227, March 1955.

(56) Lusto, L.: A capillary agglutination test for bovine Q fever. J. Immunol. 71:226–231, October 1953.

(57) Mayer, M. M., Croft, C. C., and Gray, M. M.: Kinetic studies on immune hemolysis: I. A method. J. Exper. Med. 88:427–444 (1948).

(58) Wallace, A. L., Osler, A. G., and Mayer, M. M.: Quantitative studies of complement-fixation: V. Estimation of complement-fixing potency of immune sera and its relation to antibody-nitrogen content. J. Immunol. 65:661–673, December 1950.

(59) Burton, H.: The calculation of an equivalent pasteurization time for any time-temperature of milk. Dairy Industries 16:823 (1951).

(60) Sanders, G. P., and Sager, O. S.: Phosphatase methods to determine pasteurization. *In* Standard Methods for the Examination of Dairy Products. Ed. 10. Am. Pub. Health Assn., New York, N. Y. 1953, pp. 238–256.

(61) 3-A sanitary standards for fittings used on milk and milk products equipment and used on sanitary lines conducting milk and milk products, and supplements thereto. Copies of this standard may be obtained from the Executive Secretary, International Association of Milk and Food Sanitarians, Inc., P. O. Box 437, Shelbyville, Ind.

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